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Adrenal Cortical and Other Physiological Responses to Environmental Changes in the Bovine.

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ADRENAL CORTICAL AND OTHER PHYSIOLOGICAL RESPONSES TO ENVIRONMENTAL CHANGES IN THE BOVINE

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

FIGURE Page

LIST OF APPENDIX TABLES

TABLE Page

ABSTRACT

This investigation was conducted in order to study the influence of ambient conditions on adrenal cortical and other physiological responses in the lactating bovine over a relatively long period of time. Furthermore, this investigation was designed to study the interrelationships of adrenal cortex function with certain other physiological variables.

Data were collected each month from lactating Holstein cows for one calendar year, comprising a total of 264 cow-months. The year was divided into cool, intermediate, and hot temperature-seasons. All animals were offered 100% of the estimated net energy requirements in their dietary regime. The animals were partitioned into five categories of reproductive status: pregnant 1-90 days; pregnant 91-180 days; open, normal breeder; open, anestrus; open, cycling; and 4+ services per conception.

Blood samples were collected by jugular puncture once monthly and the plasma analyzed by a competitive protein-binding method for cortisol concentration. Percents hematocrit, hemoglobin, and oxyhemoglobin were determined. Counts were made of red blood cells, total leukocytes, lymphocytes, neutrophils, and eosinophils. Blood

serum was further analyzed for total protein, albumin, total globulin, alpha$_1$, alpha$_2$, beta, and gamma globulin levels. Additionally, levels of milk production, rectal temperatures, and respiration rates were recorded.

The mean plasma cortisol concentration declined from 42.4 ng/ml in the cool temperature-season to 22.8 ng/ml in the hot temperature-season ($P<0.01$). Although not statistically significant, animals in the first 90 days of pregnancy possessed the highest cortisol levels (47.5 ng/ml) and the lowest values (29.1 ng/ml) were displayed by animals pregnant 91-180 days. A negative correlation coefficient ($P<0.01$) of -0.32 was displayed between circulating cortisol concentration and animal age.

Measurements of climatic conditions indicative of ambient heat stress were associated with a depression of hematocrit, red blood cells, hemoglobin, and oxyhemoglobin. A significant ($P<0.05$) effect of reproductive status on the number of circulating red blood cells existed. Animals in the first 90 days of pregnancy had a mean red blood cell count of 775.6 cells/cmm while anestrus animals had a mean count of 689.7 cells/cmm.

The data revealed evidence of a leukocytosis in response to increasing ambient temperature, primarily accounted for by an increase in the number of circulating neutrophils. Progression from the intermediate to the hot

temperature-season also gave evidence of an eosinophilia.

Warmer seasonal conditions promoted an increase in total serum protein values, primarily attributed to an increase in the gamma globulin fraction. Temperature-season had a significant linear effect on monthly actual milk yield ($P<0.05$) and monthly FCM production ($P<0.01$) with the highest level of milk production being obtained in the intermediate temperature-season and the lowest level observed in the hot temperature-season.

Rectal temperatures and respiration rates were elevated during the hot temperature-season ($P<0.01$). Both rectal temperature and respiration rate were negatively ($P<0.01$) associated with cortisol level.

Furthermore, plasma cortisol concentration showed a significant ($P<0.05$) correlation coefficient of 0.15 with hematocrit value and a significant ($P<0.01$) correlation coefficient of 0.25 with hemoglobin value. Increased adrenal cortex activity promoted a leukocytosis and an eosinopenia.

The data revealed an apparent negative association between cortisol level and total serum protein, total globulin, $alpha_1$, and beta globulins. However, cortisol level was positively associated with serum albumin, $alpha_2$, and beta globulin.

It is suggested that prolonged heat stress reduces

adrenal glucocorticoid secretion in the lactating bovine. It is hypothesized that this is an attempt to offset the calorogenic effect of the adrenal glucocorticoids and aid the animal to adapt to an elevated ambient heat load.

I. INTRODUCTION

Modern research in the physiology of stress was initiated by Selye's conception of the general adaptation syndrome. The study of the physiology of stress offers a stimulating challenge for basic research. Furthermore, it is important from the aspect of applied research because of its concern with animal homeostasis. High and efficient levels of animal production require a state of physiological homeostasis.

Animal production in many areas of the world, including the Gulf Coastal Region of the United States, is hindered by thermal stress resulting from high ambient temperatures. Since it is known that the adrenal cortex is involved in metabolic regulation and stress adaptation, research in this area is justified. However, the role of the adrenal cortex and its hormones under conditions of environmental stress has not been well defined in bovine physiology.

In the past, investigators have lacked sensitive and reliable methods of measuring adrenal cortex function and glucocorticoid secretion. This deficiency has been especially prevalent in bovine research. Studies involving environmental stress conditions are often approached, using

controlled conditions in climatic control chambers, and entail sudden changes of relatively short duration. Although such an approach insures a high degree of control over the experimental conditions, it may not necessarily reflect a true picture of the physiological mechanisms involved under natural seasonal environmental conditions.

This study was designed to investigate certain physiological responses to a natural climatic environment over a relatively long period of time. The data were assimilated in a manner to account for any possible effects that age, reproductive status, stage of lactation, and milk production might have on the physiological responses.

The effects of ambient environment on adrenal cortical function were studied using the competitive protein-binding method to evaluate circulating glucocorticoid levels. Additional and associated physiological measures of hematocrit, red blood cells, leukocyte profile, hemoglobin, oxyhemoglobin, blood protein fractions, milk production, rectal temperature, and respiration rate were obtained.

II. REVIEW OF LITERATURE

A. Anatomy and Histology of the Adrenals

The first gross anatomical description of the adrenal (suprarenals) glands was made by Eustochius in 1563 (58, 59). However, these observations probably lacked precision because post-mortem examinations were often delayed, due to public condemnation of such a mode of scientific inquiry, and the adrenal glands underwent rapid autolysis (58). Cuvier, in 1805, was the first observer to recognize that each gland consisted of an inner and an outer region (77).

Modern observations have shown that in the mammal, there are two capsule-covered adrenal glands and they lie at the superior poles of the two kidneys (22, 66, 77). Each gland is a composite of two distinct parts, the outer light yellow cortex and the darker, brownish inner medulla. Embryologically, the outer cortex is derived from mesoderm while the inner medulla has an ectodermal origin (77).

The cortex is composed of cords of cells with reticular tissue and capillaries between the cords. From a histological standpoint, the cortex is divided into three layers: the zona glomerulosa, zona fasciculata, and zona reticularis (66, 77).

Outermost is the zona glomerulosa. In this zone the cords are so arranged that ovoid groups of cells are formed. These cells are columnar and have deeply staining nuclei. Their cytoplasm often takes a light basic stain and they contain a few lipoid droplets.

The middle zone of the adrenal cortex is the zona fasciculata, and the composing cords are usually two cells wide and are cuboidal and frequently binucleate. These cells are filled with lipoid droplets of cholesterol, fatty acids, and neutral fat (66).

Cell cords following a non-regular course and forming a network compose the zona reticularis, which is the innermost of the three zones. The cords are one cell in width and contain large amounts of pigment. These cells have deeply staining nuclei and the cytoplasm varies in staining affinity from very light to intent (66). While susceptible to injury, the cells of the cortex can regenerate from functional cortical tissue (32, 64, 90).

Adrenal medulla cells are ovoid in nature and occur in groups. Theycontain fine granules denoted as chromafine cells. Although the cellular arrangement appears to be random, each cell has a pole directed towards a vein and a pole directed towards a capillary (66).

The adrenal glands are among the most vascular of mammalian organs. Three sets of branches arise from the larger arteries in the capsule. One of these branches

forms the capsule capillaries. A second set forms capillaries which supply the cortex and then empty into veins of the medulla. Arterial branches of the third set run through the cortex to the medulla. Veinules of the latter two sets empty into the central vein of the medulla which in turn drains the gland (65).

An abundant nerve supply for the adrenals is derived from the sympathetic branch of the autonomic nervous system. Some of the fibers are distributed to the cortex but most pass into the medulla (65).

B. General Physiology of the Adrenal Glands

The adrenal medulla secretes the hormones epinephrine and norepinephrine. These two hormones are secreted in response to sympathetic nervous system stimulation. In turn, epinephrine and norepinehprine are able to cause almost identical effects in all parts of the body as direct stimulation of the sympathetic nerves and are thus called sympathomimetic (22).

These two medulla hormones act in coordination with the sympathetic nervous system to increase the function of many body activities, including an increase in arterial pressure, blood supply to tissues, increased cellular metabolism, increased blood glucose, and increased blood coagulation. These functions enable an organism to sustain increased physical activity and thus meet emergency

situations (22).

As opposed to the two hormones of medulla origin, the mammalian adrenal cortex has been found to secrete nearly 50 hormones, which are steroidal in nature. Turner (77) states that even more hormones of adrenal cortex origin may be found as procedures and techniques for isolation and detection improve. There are two major groups of adrenocortical hormones: the mineralocorticoids and the glucocorticoids.

The mineralocorticoids are so named because of their physiological effectiveness in regulating the relative concentrations of electrolytes in the extracellular fluids, principally sodium, potassium, and chloride (22, 86). Guyton (22) states that aldosterone exerts at least 95 percent of all the mineralocorticoid activity of the adrenal cortex, but that corticosterone and deoxycorticosterone also show significant mineralocorticoid activity.

The basic functions of the mineralocorticoids are to increase renal tubular reabsorption of sodium and to increase renal excretion of potassium. Absence of mineralocorticoid regulation brings about decreased concentrations of extracellular sodium and chloride and a reduction in the total extracellular fluid volume, with an ultimate result of diminished cardiac output, a state of shock, and death. Therefore, the mineralocorticoids are necessary

for maintaining life (22). Selye (59) has designated the mineralocorticoids as the pro-inflammatory corticoids because they tend to promote an inflammation reaction to localized tissue damage.

Adrenal glucocorticoids are important in aiding the organism to resist stress. They exhibit an important effect in gluconeogenesis and bring about a decreased rate of glucose utilization by the cells. Further, the glucocorticoids cause a reduction in stored cellular protein (with the exception of the liver). This results from a decreased protein synthesis, accompanied by an elevation of circulating blood amino acids by way of mobilizing amino acids from the tissues. Fatty acids are mobilized from adipose tissue and their utilization for energy is increased. Further, the glucocorticoids promote a shift of cellular metabolism from glucose to fatty acids as a source of energy (22).

The adrenal glucocorticoids are anti-inflammatory because they inhibit the inflammation defense reaction to localized tissue damage (59). This fact has been demonstrated in the effective clinical treatment of rheumatoid arthritis with the glucocorticoid cortisone (21).

Small amounts of estrogenic and androgenic hormones as well as progestogens are secreted from the adrenal cortex (58, 77). However, the physiological importance of these compounds of adrenal origin has not as yet been generally

defined. Additionally, a large number of apparently inactive steroids may be found in the adrenal cortex which may be precursors or metabolites of active hormones (77). All ensuing discussion will pertain to the hormones of the adrenal cortex unless otherwise noted.

Classical endocrinology has shown that increased adrenal cortex secretion of glucocorticoids is due to stimulation by adrenocorticotropic hormone (ACTH). This hormone has been isolated from the adrenohypophysis and is a polypeptide having a chain length of 39 amino acids (22). It has been reported that stress elicits a release of ACTH from the pituitary (22, 59, 77). It is postulated that hypothalamic stimuli cause a release of a corticotropin release factor from the median eminance of the hypophysis, which is in turn carried by the hypophyseal portal system to the adenohypophysis sinuses and then excites ACTH secretion (22, 89). There is a general agreement that blood glucocorticoid levels are controlled by a negative feedback mechanism involving ACTH. When blood glucocorticoid levels are low, increasing amounts of ACTH are released, and reduced when glucocorticoids levels in the blood are elevated (77). Whipp et al. (85) have reported that ACTH injection stimulates the secretion of the glucocorticoids, corticosterone and hydrocortisone in Holstein calves. This work has been confirmed by Shaw and Nichols

(62). Wegner and Stott (83) have demonstrated a significant elevation of plasma corticoids within 15 minutes after an injection of 250 International Units (IU) of ACTH in Holstein heifers. Gwazdaushas and Thatcher (23) have shown an increase in the peripheral plasma concentrations of adrenocortical hormones after injection of 200 IU ACTH in the mature female bovine. A review by Yates and Urguhart (91) points out that hypophysectomy reduces adrenal secretion of adrenocortical hormones, while ACTH replacement elevates these levels. Furthermore, it has been reported that ACTH administration promotes the anti-inflammatory response attributed to increased adrenal glucocorticoid secretion in the human (21).

C. Stress

Hans Selye of the University of Montreal is credited with initiating investigations into the nature of the biology of the stress syndrome (14). In general, the word "stress" is defined as a stimulus or succession of stimuli of such magnitude as to tend to disrupt the homeostasis of the organism when the mechanism of adjustment fails or becomes incoordinate. Thus, the causative agent is not a stress, but the stimuli elicited by it is the stress. Selye (59) has adopted the term "stressor" to be used for the causative agent. However, for practical communication, the word "stress" may be used to include

both the causative agent and the stimuli which it brings about.

In the late 1930's, Selye noticed that animals under a stressful situation always exhibited a certain pattern of physiological responses, regardless of the causative agent or conditions responsible. This series of responses is known as the general adaptation syndrome and manifests a triphasic pattern. These three phases are designated as the alarm reaction, the stage of resistance, and the stage of exhaustion (42, 59).

The alarm stage of the general adaptation syndrome is the sum of all non-specific systemic phenomena elicited by sudden exposure to stimuli to which the organism is not adapted quantitatively or qualitatively. Under measurable experimental conditions, certain chemical changes in the tissues and body fluids characterize the alarm reaction. Both blood sugar and chloride ions fall to subnormal concentrations. Next, both of these entities rise to levels above normal. These two periods are known, respectively, as the shock phase and the counter-shock phase of the alarm reaction (42, 59).

Other physiological changes corresponding to the alarm reaction may include hypothermia, hypotension, hemoconcentration, acidosis, and depression of the nervous system, associated with a condition of shock. In a short

time the values for these characteristic signs may return to normal or even above normal in countershock (42).

Selye (59) further observed that in counter-shock there is an enlargement of the adrenal cortex with an involution of the thymus. During the alarm reaction, the adrenals are rapidly depleted of their lipid cholesterol and ascorbic acid content. Further, it has been demonstrated that the animal's resistance is at a very low level during the alarm reaction phase of the general adaptation syndrome.

Continued stress at a sublethal level brings about the stage of resistance, which is the second phase of the general adaptation syndrome. It has been observed that at this time the adrenal cortex returns to a normal size and the thymus regains its mass. Also, there is a change in blood sugar and chloride levels toward a normal or above normal concentration (59). The lipid content of the adrenals which was depleted during the alarm reaction also returns to normal or above normal during the stage of resistance.

During the stage of resistance the organism has reached an adaptation for the particular stress in a manner which, as yet, is not completely understood. However, this adaptation is not permanent, but is of a transient nature (59). Constantinides (14) points out that while adaptation

to a particular stress is realized in this phase, the organism's resistance to other types of stress is greatly reduced. It can be demonstrated that when an animal has reached the stage of resistance involving a particular stress, the removal of this stress and application of another type of stress will result in immediate death. Thus, Selye (59) defines the stage of resistance as the sum of all non-specific systemic reactions which ultimately develop by prolonged exposure to stimuli to which the organism has acquired adaptation as a result of continuous exposure.

If the stress is maintained upon the organism, the stage of resistance finally gives way to the stage of exhaustion. Here, the adrenals again enlarge and their lipid content turns again toward depletion. The thymus loses its regained mass and the blood sugar and chloride levels take a drastic drop. Thus, the stage of exhaustion is very much like the alarm stage as far as these symptoms are concerned. Finally, in the stage of exhaustion, the body defenses collapse resulting in death (14, 59).

Selye (59) defines the stage of exhaustion as the sum of all non-specific systemic reactions which ultimately develop as the result of very prolonged exposure to stimuli to which adaptation has been developed but could no longer be maintained.

Although certain and specific physiological changes

have been elucidated for the general adaptation syndrome, these correspond with, but are not in themselves responsible for the general adaptation syndrome. Adaptive energy is in some way mobilized and utilized to overcome the effects of stress in the alarm reaction. It is also employed to fight the effects of stress and render the animal toward a state of homeostasis in the stage of resistance. While a removal of the stress at any point during either the alarm stage or the stage of resistance will result in recovery of the animal, after the stage of exhaustion is reached nothing can prevent death. The stage of exhaustion represents a depletion of the store of adaptive energy. Also, since the stage of exhaustion is irrevocable once reached, this suggests that the supply of adaptative energy is finite and cannot be replenished.

Such factors as the nature and severity of the stressor, previous condition, history of the animal and species and certain innate characteristics of a particular animal all may contribute in determining the length of time involved and the degree of pronouncement manifest in the general adaptation syndrome. In fact, because of a lack of adequate analytical techniques, this syndrome may, at times, be recognized only under ideal experimental conditions.

Adaptation may be defined as that state of an

organism characterized by increased resistance to stress through previous exposure to stress (55). As has been previously noted, the adrenocortical hormones are secreted in response to stress. Selye (59) pointed out that the three phases of the general adaptation syndrome are paralleled by a characteristic pattern of adrenal cortical secretion (Figure 1). This observation may be verified by a determination of adrenal corticoids in the blood or by other observed physiological changes indicative of increased cortical activity.

It has been recognized that a local adaptation syndrome is exhibited when stress is confined to only one part of the body. The local adaptation syndrome is similar in effect to the general adaptation syndrome with the exception that exhaustion does not necessarily result in the death of the organism (55).

Sayers (55) has classified the responses of the adrenal cortex to stress in the following manner:

1. A sudden and temporary period of stress evokes a sudden and temporary increase in pituitary, adrenocorticotropic activity with a corresponding change in adrenal cortex secretory pattern;
2. Gradual environmental changes, such as seasonal changes, result in a gradual increase in the

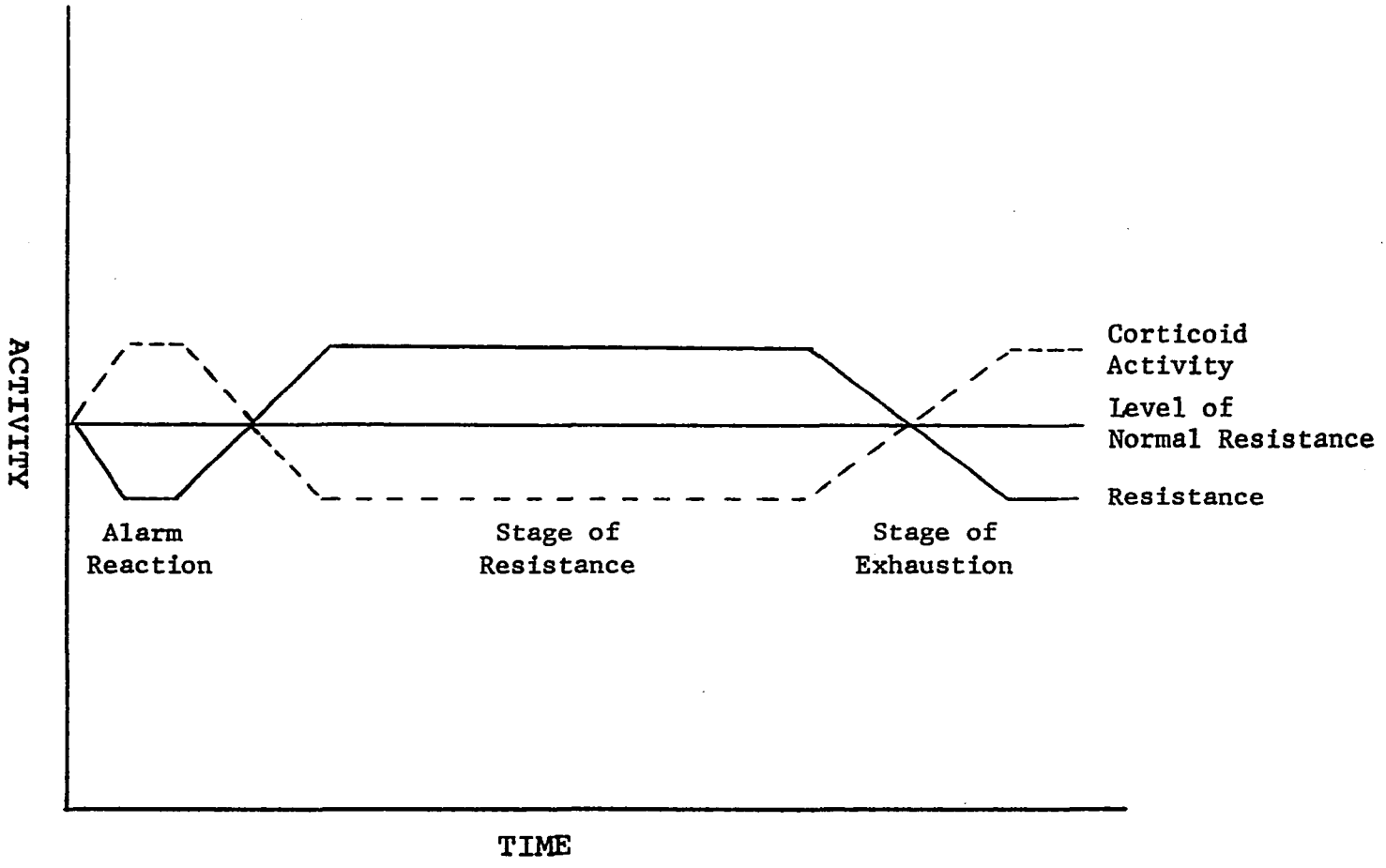


Fig. 1. Characteristic pattern of corticoid activity and resistance to stress in the general adaptation syndrome.

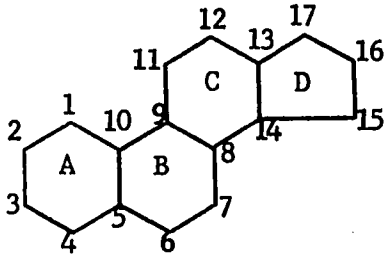
demand for adrenocortical hormones. However, the adrenal cholesterol and ascorbic acid levels remain relatively unchanged;

3. Intense continuous stress brings about a rapid fall in the adrenal content of cholesterol and ascorbic acid, with these levels remaining low until death, and;
4. If an animal adapts to stress, there is an initial depletion of adrenal constituents. However, the need for adrenocortical hormones is reduced as specific adaptations are made, and the adrenal glands return to a pre-stress stage of activity.

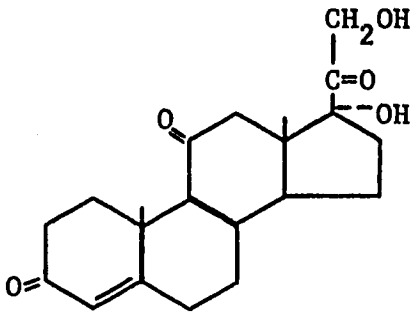
It was further stated that "A most striking feature of the adrenal cortex as an organ of homeostasis, is its ability to endow the organism with resistance not to a few, but to all types of stress."

D. Synthesis of the Glucocorticoids

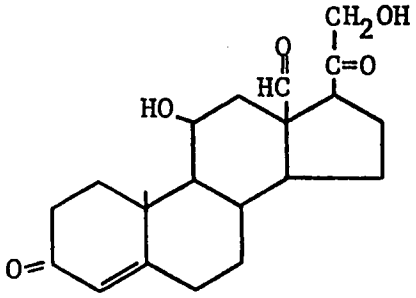
Biochemically, the adrenal cortical hormones are steroidal in nature. It has been previously stated that the adrenals contain a high concentration of cholesterol, and the adrenal steroids may be synthesized in vivo from either cholesterol or acetate (86). Figure 2 shows the basic steroid structure and the structure of six of the most active glucocorticoids.



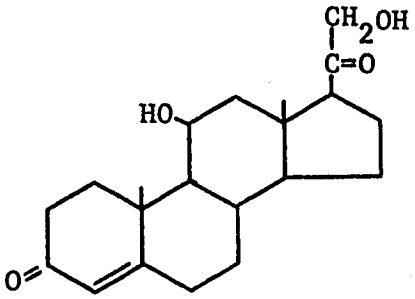
Steroid nucleus



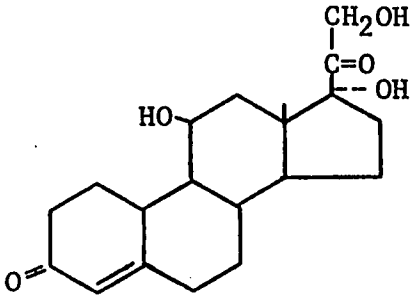
Cortisone



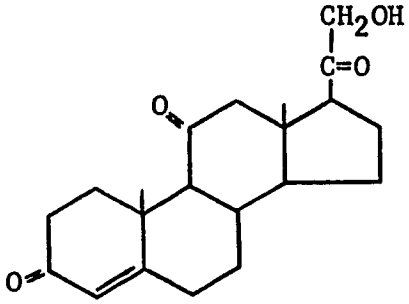
Aldosterone



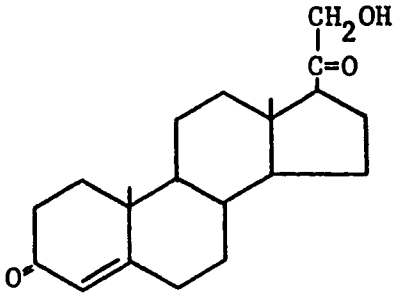
Corticosterone



Cortisol



11-Dehydrocorticosterone



Deoxycorticosterone

Fig. 2. Steroid nucleus and some of the active glucocorticoids.

Evidence exists to indicate that pregnenolone plays a role in the metabolic synthesis of adrenal steroids. Fevold (19) found that incubated rabbit adrenal homogenates possessed the ability to utilize either pregnenolone or progesterone as an exogenous substrate for the in vitro synthesis of cortisol. In this work, the total amount of cortisol formed was the same with each of the substrates, but pregnenolone substrate showed a greater efficiency for conversion into cortisol. However, Cameron and Griffiths (10) studying the transformation of radioisotope labelled pregnenolone and progesterone into cortisol by human adrenal cells incubated in vitro, found that both of these compounds serve as precursors for a variety of corticosteroids and intermediates. Additional work suggested that the relative amount of pregnenolone transformation into cortisol might be slight when the transformation into 16 alpha-hydroxyprogesterone, 17 alpha-hydroxyprogesterone, and 11-deoxycorticosterone were evaluated by $^{3}H:^{14}C$ ratios (9). Under these experimental conditions, it was concluded that progesterone was not necessarily an intermediate for the conversion of pregnenolone into cortisol (41).

Holzbauer and Newport (27) analyzed extracts from rat adrenals by gas-liquid chromatography and concluded that an acceleration of pregnenolone and progesterone

synthesis is a mandatory prerequisite for increased corticosterone synthesis. This further suggests a definite involvement of pregnenolone and progesterone as precursors of corticoid synthesis.

Other investigators (31) have reported that adrenal homogenates rapidly convert progesterone to deoxycorticosterone, but that conversion of progesterone to corticosterone is poor. However, the relative efficiency of the conversions in these experiments may have been abnormally altered by previous treatment of the animals by methylandrostenediol.

Sandor and Lanthier (54) demonstrated that both bovine and human adrenal glomerulosa tissue are capable of synthesizing 18-hydroxycorticosterone from progesterone under in vitro conditions. The conversion yield was found to be 3.8% and 0.5%, respectively, for the two tissues. Wagner et al. (82) determined a highly significant correlation between adrenal progesterone and adrenal cortisol in cows. These data suggest that progesterone may be an obligatory intermediate in adrenal cortisol synthesis. Gwazdaukas et al. (24) state that in the bovine, adrenal progesterone is regarded primarily as a precursor for the synthesis of both gluco- and mineralocorticoids.

Results from in vitro research further point out the probability of adrenal glucocorticoids acting as

precursors for the metabolic synthesis of other adrenal glucocorticoid steroids. Both bovine and human adrenal tissue are capable of producing 18-hydroxycorticosterone from corticosterone (54). Bovine adrenals can utilize deoxycorticosterone as a substrate for the production of corticosterone. Also, 11-deoxycortisol is capable of *in vitro* transformation into cortisol (21).

It has been suggested that aldosterone, which has both a mineralo- and glucocorticoid effect is secreted by the adrenal cortex *zona glomerulosa* and that the *zona fasciculata* is responsible for glucocorticoid secretion. The *zona reticularis* may have the secretions of the adrenal sex hormones as its principal function (22). Prolonged ACTH stimulation produces hypertrophy of both the *zona fasciculata* and the *zona reticularis*. Absence of ACTH secretion results in atrophy of these two zones, while leaving the *zona glomerulosa* intact.

This viewpoint has been partially substantiated by findings that pregnenolone conversion to glucocorticoids, particularly cortisol, was significantly higher in vascicular tissue than in reticular tissue by human adrenal *in vitro* incubations. However, results with progesterone substrate did not reveal any differences between the *zona fasciculata* and the *zona reticularis* (9).

A review by Yates and Urguhart (91) notes that the

mammalian adrenal zona glomerulosa appears to be the source of aldosterone, and both the zona fasciculata and the zona reticularis are sources of cortisol, while corticosterone is produced in all three zones. Wagner et al. (82) report that the zona fasciculata and the zona reticularis are both assumed to be cortisol producing areas in the bovine.

There is evidence that ACTH plays a role not only in the stimulation of adrenal cortical hormone secretions, but also as a factor in regulating the metabolic synthesis of these hormones. It has been demonstrated that rabbit adrenal homogenates are capable of in vitro synthesis of cortisol if the homogenates have been pre-stimulated for 28 days with ACTH. Cortisol was not synthesized by tissues which were not pre-stimulated with ACTH (19). Additionally, it has been surmised that prior treatment with ACTH exaggerates the response of plasma corticoid concentration to ACTH infusion. This indicates that ACTH may increase adrenal response to further ACTH stimulation (91).

It has been shown that the magnitude of the response to ACTH stimulation of circulating levels of cortisol is greater than corticosterone in the intact bovine (24). Other workers (79) have verified this finding, and attribute this differential response to the preferential stimulation of ACTH on the 17-hydroxylating system of the adrenal gland which leads to cortisol

synthesis while simultaneously decreasing the substrate available for corticosterone synthesis.

Figure 3 shows some of the possible biochemical pathways of adrenocorticoid synthesis. The following points concerning the molecular configuration of these compounds have been set forth by Turner (77):

1. A double valance bond at carbon four is necessary for corticoid activity;
2. Ketonic groups (C=0) at carbons three and twenty are necessary for corticoid activity;
3. A hydroxyl group (OH) at carbon 21 enhances sodium retention and is necessary for carbohydrate metabolism;
4. A hydroxyl group at carbon 17 heightens the effects on carbohydrate metabolism and;
5. An oxygen (either O or OH) at carbon 11 exerts major effects on carbohydrate metabolism and usually decreases the ability for sodium retention, with the exception of aldosterone.

E. Duration of Glucocorticoid Activity

A review by Yates and Urguhart (91) reveals that corticosteroids in the circulating blood may exist in three forms; free (native), bound to albumin, or bound to corticosteroid-binding globulin. The blood protein albumin is a

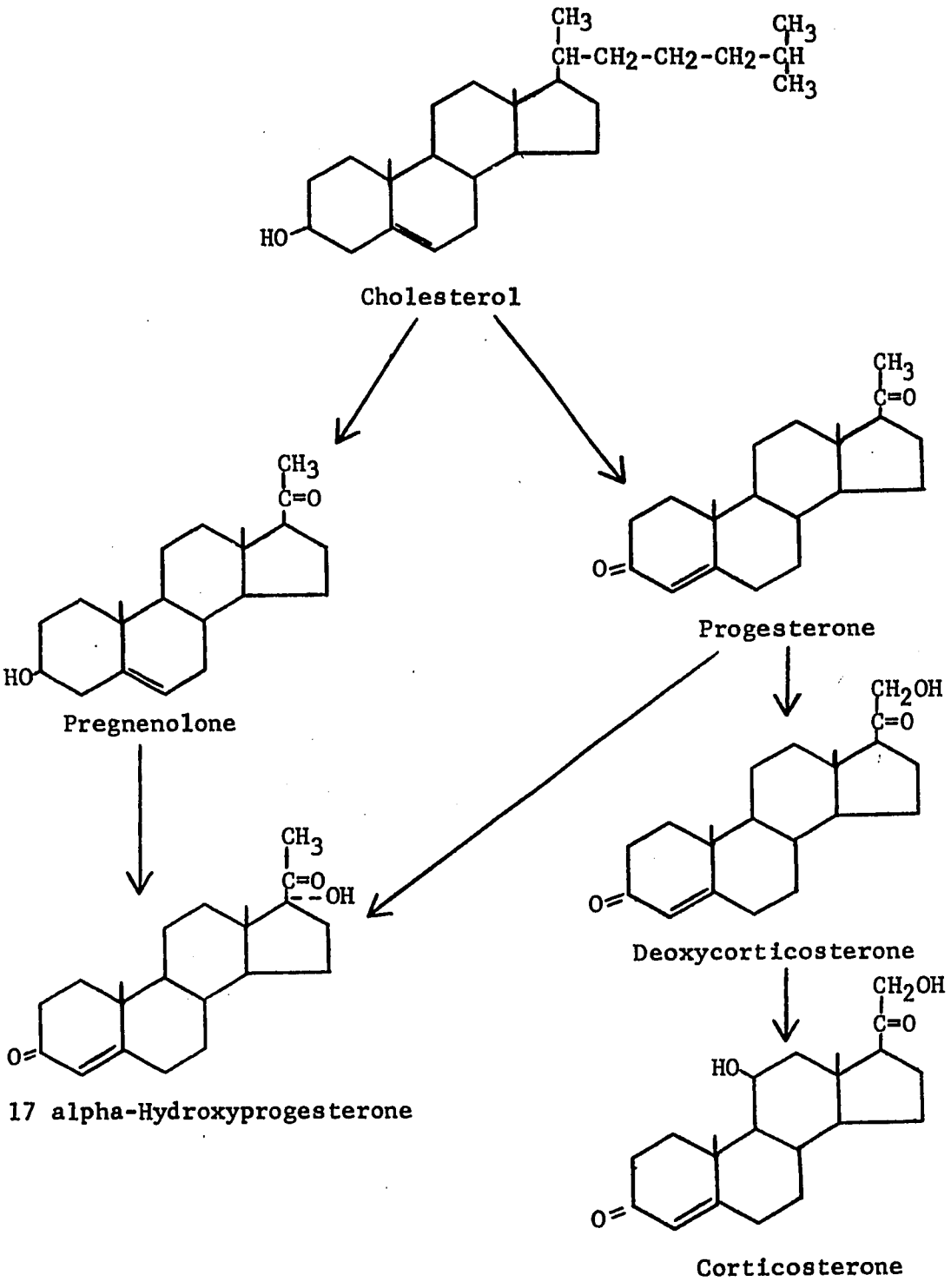


Fig. 3. Some possible pathways of adrenocorticoid synthesis.

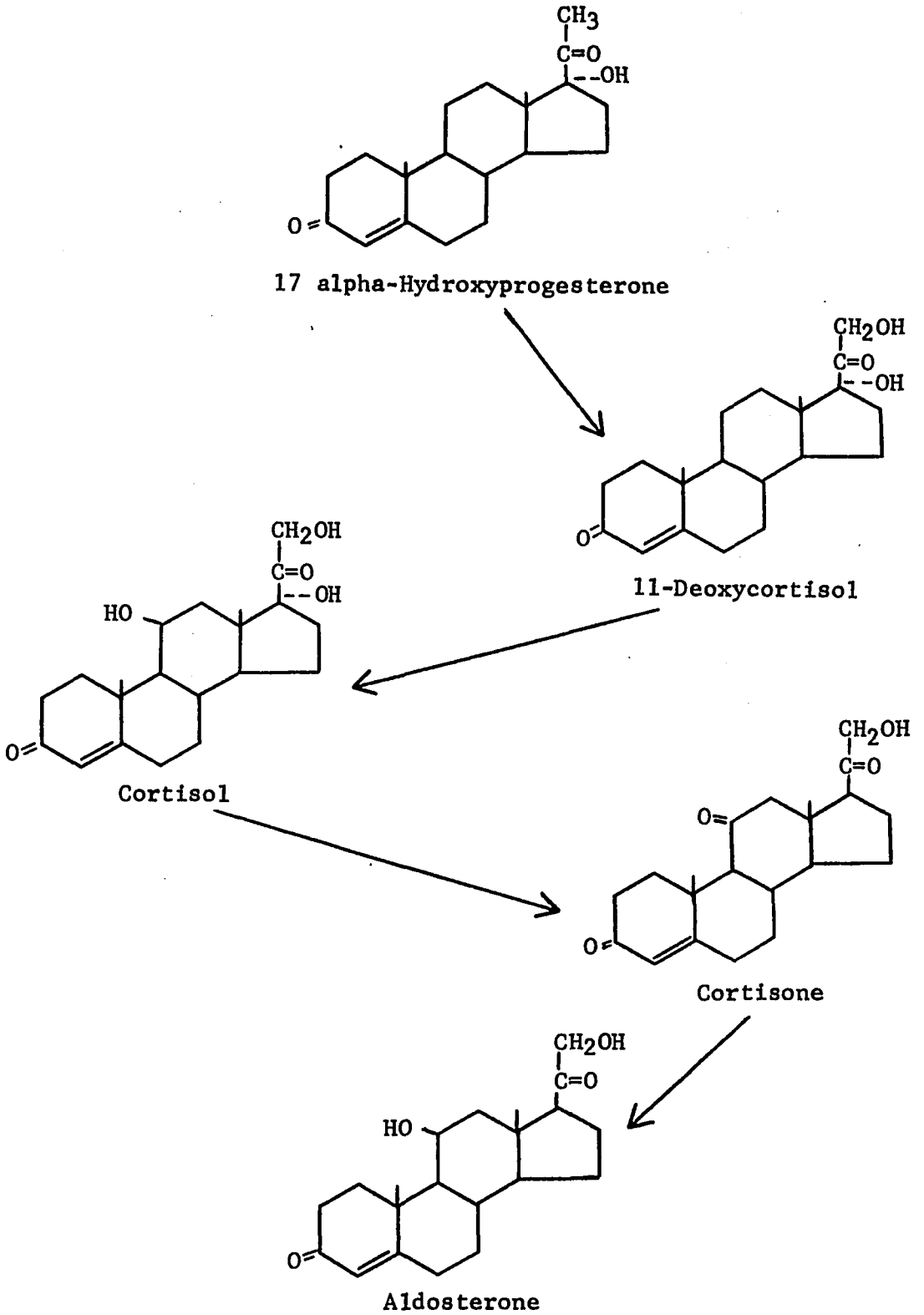


Fig. 4. A possible pathway of adrenocorticoid synthesis.

binding agent having low affinity but a high binding capacity for corticosteroids. Conversely, corticosteroid-binding globulin (CBG, transcortin) has a high affinity for the binding of corticosteroids, but its binding capacity is comparatively low. Seal and Doe (56) found evidence for the existence of corticosteroid-binding globulin in 131 animal species representing all vertebrate classes.

Under normal conditions, corticosteroid-binding globulin is more nearly saturated with corticosteroids than is albumin, because of the higher affinity of the former for corticosteroids. And, since albumin has a low affinity for corticosteroids, any rapid ACTH activity will show a disproportionately larger increase in the active or free corticosteroid concentration (91).

The effective duration of circulating glucocorticoids has been found to vary with species. The specific activity of tritium labelled cortisol, administered to sheep by intravenous infusion, fell in an exponential manner to a non-measurable amount in 20 to 40 minutes (47). Additional experimentation confirmed this rapid disappearance of cortisol and revealed that the decrease in plasma cortisol concentration was even more rapid in ewes in the last two weeks of pregnancy (49). This change in the velocity of decrease was apparent at five minutes post-infusion. This latter finding may have been due to an

expansion in plasma volume at this stage of pregnancy or it may have been in some way associated with the initiation of fetal adrenal cortisol secretion.

The elevation of plasma corticosterone levels in the rat in response to stress is rapid. This is exemplified by the fact that rats must be rapidly decapitated in order to obtain "resting" plasma corticosterone values (32). Plots of plasma corticosterone levels versus time after stress show an immediate increase in plasma levels of steroid (64). Research has shown the mean maximum plasma concentrations of corticosterone in the rat to occur approximately one hour after stress or after subcutaneous injection with the steroid (65). It was also revealed that although variable dose levels of ACTH (1 IU and 8 IU) gave a difference in the magnitude of circulating corticosterone levels, the major effect was found in a greater persistency of activity with the higher level. However, regardless of the ACTH dose level, approximately 24 hours were required for the plasma corticosterone values to return to the pre-injection levels. These findings in regard to elevation and persistency were validated by experiments employing subcutaneous corticosterone injections (65).

Balfour (3) reported that ACTH administration to calves less than eight days of age exhibited no adrenal

effects. Between the ages of eight and 40 days, ACTH administration reportedly did not increase the concentration of hormones in the blood, but only increased the amount of blood leaving the adrenal gland, as measured by a drop counter.

However, Wegner and Stott (83) found that ACTH administration to 18-month-old Holstein heifers gave a highly significant increase in plasma corticoids from 7.9 ng/ml to 43.9 ng/ml in 15 minutes. These animals displayed a peak of 57.4 ng/ml of circulating corticoids in 30 minutes which continued for two hours. Pre-injection levels were noted at eight hours.

In an experiment involving mature cows, Brush (7) observed that a 200 IU injection of ACTH produced a rapid increase in circulating 17-hydroxycorticosteroids. This was followed by a rapid drop and then a more gradual fall to normal levels in 24-48 hours. Chromatography of the plasma corticoids showed cortisol to be the only detectable corticoid present.

Other workers have confirmed these findings. Venkataseshu and Estergreen (79) measured the response of lactating, non-pregnant dairy cows to an injection of 200 IU ACTH and found that this treatment more than doubled the concentration of cortisol (4.1 to 8.9 ng/100 ml) by one hour after administration, resulting in highly significant

treatment effects between hours post-treatment. Cortisol concentration began to decline by the second hour post-treatment and was at 127% of pre-injection levels at three hours post-injection. ACTH treatment did not elevate circulating corticosterone levels. In fact, corticosterone levels held nearly constant the first hour post-injection and then declined to a low of 52% at two hours post-injection.

Other workers (23, 24) likewise report an immediate increase in circulating cortisol levels due to a 200 IU injection of ACTH in Holstein cows. An increase from 3.1 ng/ml 60 minutes pre-injection to 5.9 ng/ml five minutes post-injection was obtained. An average peak of 45.8 ng/ml was obtained at 30 minutes post-injection and these high levels were maintained for the duration of the four-hour sampling period. It is interesting to note that ACTH also gave a corresponding response in circulating corticosterone levels, although a lower magnitude of 0.1 ng/ml at 60 minutes pre-injection and a 30-minute peak of 7.6 ng/ml. These findings in regard to corticosterone are not in agreement with those of the work reviewed above (79).

A review by Yates and Urguhart (91) in 1962 gave evidence that diurnal variations for the total plasma concentration of corticosteroids existed in man, monkey, dog, rat, and mouse. Dorfman (15) states that adrenocorticoid analysis of human blood and urine shows a diurnal

cycle of approximately 24 hours in regard to adrenal secretion. This pattern of diurnal variation has been observed to be upset by the stress of surgery. However, no diurnal pattern was detected in the bovine, possibly because of relatively low levels of circulating corticosteroids in cattle and the inefficiency of identification and measurement of these compounds at that time. Some years later, Wagner (81) using the more sensitive protein-binding method reported findings which did indicate a diurnal effect in cows. He reported a significantly lower level of plasma corticoids in the 1600-2400 hour period (5.6 ng/ml) than in the 2400-0800 (7.2 ng/ml) and 0800-1600 (7.5 ng/ml) hour time periods.

F. Levels and Ratios of Glucocorticoids

Hyde and Skelton (32) report the plasma corticosterone level of unstressed rats to range from 3.0 to 7.0 ng/100 ml. In this work the values were obtained by isotopic dilution and under rigidly controlled conditions to insure a "resting" state.

The ratio and levels of cortisol to corticosterone in cat adrenal venous blood was studied by Ilett and Lockett (33). Their work revealed that cortisol varied from 0.69 ng/ml in the neuter kitten to 6.65 ng/ml in the neuter adult. The lowest corticosterone level was 0.53 ng/ml in the adult female and the highest level was 4.07

ng/ml in the neuter adult. It was concluded that the cortisol to corticosterone ratio is less than unity for sexually immature cats but this ratio increases with age. Other investigators (28) found that puppies secreted more cortisol than did adult dogs on a body weight basis. Furthermore, it was determined that in adrenal venous blood of young pigs and dogs cortisol and corticosterone were the principal steroids present and comprising a total of 64% of the total steriods.

Research findings indicate that the circulating levels of cortisol are at a higher level than corticosterone in the mature bovine. Estergreen and Venkataseshu (17) reported a cortisol to corticosterone ratio in lactating cows varying from 0.85 to 5.94, with a mean of 2.40. Gwazdauskas et al. (24) found that circulating cortisol levels in Holstein cows ranged from 3.1 to 8.8 ng/ml, while corticosterone levels ranged from 0.5 to 1.1 ng/ml. Other workers (79) report a low cortisol:corticosterone ratio of 1.5:1 for Guernsey cows and a high ratio of 4.0:1 for Holsteins.

<u>In vitro</u> perfusion of cow adrenals with ACTH produced hydrocortisone and corticosterone in ratios from 4:1 to 1:1 (26). However, these workers reported that while steer adrenals produce some corticosterone, there is little or no production of hydrocortisone. It is therefore indicated

that castration or sex differences may influence adrenal enzymatic systems in such a way as to alter the components of secretory product.

Thompson et al. (76) observed plasma levels of 17-hydroxycorticosterone to range from 1.7 to 4.5 micrograms percent in Holstein heifers with the higher level being related to higher environmental temperatures. Whipp et al. (85) found a cortisol to corticosterone ratio of 5:8 in young Holstein calves stimulated by ACTH infusion. These researchers concluded that steroid values in young calves were too low for measurement without the benefit of ACTH stimulation. However, Purohit and Estergreen (50) using a more sensitive double isotopic dilution derivative assay found the mean plasma cortisol and corticosterone levels in young dairy calves to be 7.2 and 0.7 ng/100 ml, respectively, at one day of age. This gave a cortisol to corticosterone ratio of 13.0:1. With increasing age the cortisol decreased while corticosterone increased to give respective values of 4.9 and 2.8 ng/100 ml at 16 days of age. These changes resulted in a reduced ratio of 1.9:1. It was postulated that the gradual reduction in cortisol during the first 16 days of life of the calves may indicate that the calves were recovering from stress of birth and were adjusting to the stress of their postnatal environment.

G. Degradation of Glucocorticoids

The liver is the principal site of degradation of the glucocorticoid hormones. Experimental work shows that the rate of cortisol secretion is unchanged in the ewe in advanced stages of pregnancy but that the concentration of unbound plasma cortisol is decreased (48). This indicates there is either an increase in the efficiency of extraction of cortisol by the liver or an increase in the amount of tissue, which clears cortisol as the end of term is approached. It may also be that near term the fetal liver metabolizes significant amounts of cortisol of maternal origin.

Brown et al. (5) followed the disappearance of injected ^{14}C-labeled steriods from sows and concluded that the primary excretion route is through the urine. It was further observed that the metabolism of cortisol is more rapid than corticosterone in the sow. These observations may suggest that the corticosterone molecule may undergo more transformations before it can be excreted than does the cortisol molecule.

Research performed by Willett, Brown and Erb (88) involved isolation and tentative identification of ^{14}C-labeled corticosteroid metabolites from the urine of an ovariectomized heifer. These workers recovered 11.7% of the injected radioactivity from cortisol and 7.4% from

corticosterone from the urine at 12 hours. At 72 hours, 29.0% and 20.2% of the radioactivity from cortisol and corticosterone, respectively, was recovered from the urine. This indicated that cortisol degradation is more rapid than corticosterone degradation in the bovine.

While absolute identification of the metabolites was not possible due to small quantities, tentative identifications and percents as determined by column and paper chromatography are presented in Table 1.

Table 1. Tentative identification and percent of degradation products of cortisol and corticosterone (88).

Compound	Cortisol	Corticosterone
	--------(%)----------	
6-hydroxycortisol	35.0	3.6
Tetrahydrocortisol	19.0	15.7
Cortols	6.4	2.2
Cortolones (11-keto)	4.5	2.4
Tetrahydrocorticosterone	1.6	17.6
Unknown, highly polar compound(s)	---	34.0

Table 1 reveals that corticosterone degradation yields at least one, and possibly several, unknown highly polar compound(s) not found in cortisol degradation. This may, in part, account for the relatively slower complete

degradation and urinary excretion of corticosterone as compared to cortisol.

H. Glucocorticoid Relationship to Reproduction

Adams and Wagner (1) reported an increase in the level of corticoids in the pregnant bovine one to four days prior to parturition. Their findings suggest that this abrupt increase in maternal plasma corticoids may result from increased synthesis by the fetal adrenals. It is further suggested that this increase may cause a decline in corpus luteum function and serve to initiate parturition.

Liggins (37) states that stimulation of fetal lamb adrenals by ACTH or cortisol infusion leads to premature parturition. However, infusion of large doses of cortisol into pregnant ewes was ineffective. Additional work showed dexamethasone (a synthetic glucocorticoid) caused premature delivery when infused into the fetal lambs, but not when administered to pregnant ewes. Fetal infusion with deoxycorticosterone and corticosterone, which are primarily mineralocorticoids in sheep, did not cause premature delivery (38). Thus, the ability of corticosteriods to cause premature parturition appears to depend on glucocorticoid rather than mineralocorticoid activity.

In twin fetus', hypophysectomy of both fetal lambs in vivo caused prolonged gestation. However, when one twin

had an intact pituitary, parturition occurred at term (16, 38). This indicates that the fetal pituitary adrenal axis is involved in the initiation of parturition. None of the overdue ewes showed signs of impending parturition such as mammary development, softening of sacro-sciatic ligaments, and swelling and relaxation of the vulva. With incomplete adrenalectomy, spontaneous delivery did take place at or near term after regeneration of fetal adrenal tissue. Paterson and Harrison (48) report that the rate of cortisol secretion in sheep did not change during pregnancy. But they did find a decrease in the concentration of cortisol in plasma the last two weeks of pregnancy.

It has been observed that increased or decreased body temperature of adrenalectomized pregnant rats maintained with 11-deoxycorticosterone acetate (DOCA) showed no ill effects on embryos (18). However, significant embryo degeneration resulted when intact rats were subjected to thermal changes. It has been postulated that harmful effects in intact rats were due to an increase in cortical hormones from increased ACTH secretion. Selye (57) reported ovary atrophy, promoting anestrus and adrenal cortex hypertrophy, in rats subjected to stress, leading to reproductive inefficiency.

Howarth and Hawk (29) studied the effects of exogenous hydrocortisone acetate on ovum fertilization and embryonic survival in sheep. Results showed that this

compound had no effect on fertilization, but significantly reduced embryonic survival during late summer and early fall. No effect was found during the winter months. It was hypothesized from these results that adrenal hyperactivity could be a factor in the adverse effects of stressful environmental conditions on reproduction.

According to Velardo (78), ACTH administered to rats reduced litter size and produced an increase in the number of stillbirths, with the greatest effect from ACTH injections on the day of mating and for six subsequent days. ACTH given to adrenalectomized rats did not display any ill effects. It was further found that maternal adrenalectomy prior to day seven of pregnancy caused a reduction in litter size and an increase in the stillborn. However, adrenalectomy past the seventh day had no effect. Thus, it appears that the effects of ACTH is mediated through the adrenals. It was suggested that an interaction exists between hormones of the adrenal cortex and ovaries, and is displayed on the uterus as an endocrine receptor organ (78).

Shaw et al. (61) determined from 451 measurements on 46 cattle that heifers in or near estrus and non-pregnant, lactating, in or near estrus cows had plasma 17-hydroxycorticosteroid levels of 10.3 ng/100ml and 10.8 ng/100 ml, respectively. These values were significantly

higher than those for heifers not in or near estrus (4.4 ng/100 ml) and those of lactating, non-pregnant cows not in or near estrus (4.3 ng/100 ml). Therefore, animals in or near estrus had higher 17-hydroxycorticosteroid levels but neither pregnancy nor lactation caused any appreciable elevation.

Other research presented no differences in plasma 17-hydroxycorticosteroid levels in the bovine at 33 days pre-calving and five days post-calving (6). These results in the bovine were contrary to humans in which levels of 17-hydrocorticosteroids were found to be elevated after parturition.

Stott and Thomas (75) have noted that heifers conceiving on the first service showed plasma corticosteroid levels higher than those with breeding anomalies. This phenomenon seemed to be related to the degree of stress imposed by the severity of dietary deficiency.

Other work reveals that normal cycling and conception occurs in postpartum lactating cows only when the plasma cortisol concentration varies intermittently (74). Evidence indicates that plasma cortisol concentration reflects the activity of the pituitary gonadotrophins. Apparently the mechanism controlling pituitary and adrenal releases is not independent of gonadotrophic release, specifically FSH.

I. Glucocorticoid Relationship to Lactation and Suckling

Rivera (51) observed the influence of adrenal corticosterone on the enzyme activity in mouse mammary gland *in vitro*. This worker noted that the entry of adrenal corticosteroid into mammary cells appears to be very rapid. The measured activities of glucose-6-phosphate and 6-phosphogluconate dehydrogenase revealed that prolactin, in addition to insulin, produced activity in the secretory development of the explants. However, this induced activity was more consistently maintained over a time period with the presence of corticosterone. Thus, it may be that in the presence of all three hormones there is a preferential stimulation of glucose oxidation, and adrenal corticosteroid serves to maintain rather than induce rises in enzyme activity. Other workers (41) report that the formation of rough endoplasmic reticulum in mammary explants may be attributed to adrenal corticosteroids. Presumably the importance of corticosteroid would be more important in the later stages of enzyme response.

Gala and Westphal (20) found a decrease in corticosteroid-binding globulin in rats on the third day of lactation. Furthermore, corticosteroid-binding globulin levels were found to be inversely related to the number of pups per litter. Four days after weaning the corticosteroid-binding globulin levels returned to virgin level. These

results indicate that corticosteroid-binding globulin plays an important role in the initiation of lactation.

The data of Hahn and Turner (25) shows that 0.75 mg corticosterone per rat per day decreased the average milk yield 7.5% on days 14 and 20 of lactation. It further shows that 1.0 mg gave a significant increase of 16.4%, while a corticosterone dosage of 1.25 mg per day gave a 10.8% increase in milk yield on days 14 to 20 as compared to the controls. These findings strongly suggest that corticosterone plays a role in normal lactation in the rat, and that an optimum corticosterone level for lactation exists.

Shaw et al. (61) reported in 1960 that lactation in cattle did not cause an appreciable effect in circulating 17-hydroxycorticosteroid levels. However, Smith et al. (68) in 1972 using more refined techniques measured corticoid serum concentration in Holstein cows in order to study the corticoid response to milking and exteroceptive stimuli. It was determined that serum corticoid concentration averaged 3.9 ng/ml prior to milking and was significantly increased to 8.1 and 11.5 ng/ml at 5 and 15 minutes after the start of milking. These concentrations had fallen to pre-milked values by 60 minutes after the start of milking. Non-milked pairmates yielded serum corticoid values of 4.6 ng/ml ten minutes

before the milking process was initiated and increased to 6.7 and 8.2 ng/ml at -5 and -1 minutes. By 60 minutes after their pairmates were milked, serum corticoid of non-milked cows had fallen to 3.1 ng/ml, 32% lower than at ten minutes before milking. It was concluded that the milking stimulus per se and exteroceptive stimuli can increase the circulating corticoid levels in the cow.

Wagner (81), using indwelling jugular vein cannulas, collected blood samples at 30-minute intervals to determine the effect of lactation on plasma corticoids in nine cows. Mean corticoid levels were measured by the protein-binding method and were 4.5, 6.8, and 9.4 ng/ml for non-lactating, milked, and suckled groups, respectively. The non-lactating and suckled groups were significantly different. This experiment also showed that a ten-unit dosage of vasopressin resulted in a three-fold increase in corticoid levels from 10 ng/ml to 29 ng/ml.

However, it has also been reported that plasma cortisol levels were higher, ($P<0.01$) for cows milked 30 days, over those nursed 30 days (82). Smith and Convey (67) reported that the stimulus of either continuous or intermittent suckling greatly increased the plasma corticosterone levels of rats when compared to non-suckled rats.

Other workers (46) reported that the average plasma

corticoid level for cows prior to milking was 9.7 ng/ml. Overmilked cows (milked 15 minutes by machine) had an average value of 22.0 ng/ml which was significantly greater than the level after normal machine milking of 19.5 ng/ml.

An injection of ACTH (200 IU) in cows produced a rapid increase in circulating 17-hydroxycorticosteroids, followed by a rapid drop, followed by a more gradual fall to normal in 24 to 48 hours (7). ACTH injection also produced a rapid fall in milk yield, with a return to a steady normal taking five to seven days. Other investigators (8, 60) have verified the decrease in milk production after ACTH injection in both normal and ketotic cows. This severe milk depression was apparently due to abnormally high ACTH levels.

It was found that rats suckled 30 minutes after a 12-hour period of non-suckling caused a reduction in pituitary ACTH concentration by 60% and a four-fold increase in the plasma corticosterone level (80). Suckling three hours after the non-suckling period brought about an increased pituitary ACTH concentration of 85% and a four-fold increase in the plasma corticosterone level. It was concluded that suckling stimulated pituitary ACTH release, which in turn evoked increased secretion of corticosterone by the adrenal glands. These workers (80) further assumed that, in the rat, corticosterone increases at the time of

initiation of lactation.

J. Hormone Balance

The nature of the balance and interrelationships of adrenal glucocorticoids to other hormones is an important aspect of endocrinology. Thompson et al. (76) measured plasma levels of 17-hydroxycorticosterone, serum protein-bound iodine, thyroxine utilization and secretion rates of Holstein heifers under cool and hot conditions. Although treatment imposed did have an effect on the individual measurements, adrenocortical response was not significantly related with any measure of thyroid activity.

Sinha and Schmidt (63) fed lactating rats a diet containing approximately ten times more thyroxine and triiodothyronine than the normal secretion rate of thyroxine. The feeding of this level of thyroactive material did not affect the pituitary gland weight, but did greatly increase adrenal size and increase the concentration of plasma corticosteroids. These results indicated that hyperthyrodism causes a hormonal imbalance with adrenal hormones which may result in impaired lactation.

The relationship between corticosteroids and thyroid activity in dairy cows was studied by Stockl and Jochle (72). These workers injected lactating cows with two corticoids, flumethasone (6 alpha, 9 alpha-difluoro-16 alpha-methyl-11 beta, 17 alpha, 21-triol-1, 4 pregnadien-

3, 20 dione) and dexamethasone TMA (9 alpha-fluoro-16 alpha-methyl-11 beta, 17 alpha, 21-triol-1, 4-pregnadien-3, 20 dione 21-trimethyl-acetate). Results revealed that both corticoids raised the total plasma iodine with higher levels being obtained in cows four to one week post parturition as compared to animals in late lactation. It was concluded that one of the beneficial effects of corticosteroid therapy might be due to a temporary stimulation of thyroid activity.

It has been reported that a highly significant correlation exists between adrenal progesterone and adrenal cortisol levels in the bovine (82). This correlation was arrived at from steroid determinations obtained by homogenization and subsequent thin layer chromatography of adrenal glands.

Stott and Robinson (66) reported a sharp but short-lived increase in both plasma cortisol and progesterone in dairy cattle subjected to acute thermal stress. Lee et al. (36) found no relationship between these two hormones with weekly increasing levels of thermal stress with samples being taken at the end of each week.

It is indicated that FSH secretions and possibly other gonadotropic secretions are related to an adrenal function in the bovine (75). Velardo (78) reports that data concerning the effects of ACTH on reproduction in the rat indicate that a very delicate ratio of hormones of the

adrenal cortex and ovaries is required for successful gestation in laboratory animals.

K. Blood Profile Associated with Glucocorticoids

In 1950 it was reported that lymphocytes are partially under the regulatory control of the adrenal cortex, and lymphocytopenia may result from increased adrenal cortex activity (55). The phenomenon is apparently initiated by a discharge of ACTH, stimulating release of certain cortical steroids. Neither stress nor ACTH induced lymphocytopenia in adrenalectomized animals. It was also reported that ACTH administration in man will produce eosinopenia in approximately four hours (55).

Other workers (55, 60, 69, 70) found that, in addition to an eosinopenia, the percent of circulating neutrophils exceeds the percent of circulating lymphocytes four to six hours after ACTH administration to cows. This same ratio change was observed at parturition or on the day preceding parturition. White (87) observed leukocytosis and an increase in erythrocytes and hemoglobin values in sheep stressed with an injection of formalin. In the latter stages of the experiment, the absolute lymphocyte and eosinophil counts were depressed while the number of circulating monocytes had risen. Thompson et al. (76) reported that a sudden exposure to heat stress produced a temporary eosinophilia in dairy heifers.

Paape et al. (45) reported that cows under a constant ambient temperature of 21, 27, or 32 C showed no effect in circulating erythrocyte counts. However, at 27 C or 32 C reductions in circulating erythrocytes did occur whenever night temperatures were lowered to 21 C for a 12-hour night period. This was attributed to a hemodilution effect due to consumption of large amounts of water during the higher day temperatures. The same response was found with circulating leukocytes. These workers (45) found no significant trends with changes in ambient temperature in percent neutrophils, lymphocytes monocytes, or eosinophils. However, a large cow-by-treatment interaction in the data may have masked actual changes.

Roussel et al. (53) collected data from beef bulls maintained at a constant ambient temperature of 18 C or under a natural environment with temperatures ranging from 19.4 to 34.8 C. An elevation in the percentage of white blood cells, monocytes and eosinophils was found in the bulls in the higher temperature group. Bulls in the cooler temperature group displayed an elevation in the percentage of neutrophils and lymphocytes.

Wegner and Stott (83) determined an evident leucocytosis at two hours after a 250 IU ACTH injection to 18-month Holstein heifers. This leukocytosis was statistically

significant at four and eight hours. A significant lymphopenia and eosinopenia was found in the four-hour blood samples, along with a significant increase in circulating polymorpho-nuclear leukocytes. Most of the leukocytosis was accounted for by a two-fold increase in the number of circulating neutrophils.

L. Heat Stress and Adaptation

The efficient production of milk, meat, and other products of animal origin is, to a large degree, dependent upon the physiological well-being of the animals, or on the maintenance of homeostasis. This is evident in that certain production data such as weight gains and milk production may be used as a bioassay to evaluate the effects of stressor agents (89). It was further noted that reproductive efficiency can be affected by stress (29, 74).

Howarth and Hawk (29) reported that exogenous cortisol acetate had no effect on fertilization in sheep, but significantly reduced embryonic survival during late summer and early fall. However, their research disclosed no ill effects of the corticoid during the winter months. It may be hypothesized that adrenal activity may be a factor in the adverse effects of stressful environmental conditions on reproduction. Thus, it seems that an interaction exists between adrenal activity and stressful

environmental conditions which affects reproduction.

Other workers (27) report that a short-term (5 to 15 minutes) exposure of rats to cold stress gave increases in adrenal concentration of pregnenolone, progesterone, and corticosterone. Acute thermal stress, according to Stott and Robinson (74), produces a sudden but short-lived large increase in both circulating cortisol and progesterone in the bovine while continued stress suppressed the levels of both hormones.

Rats were exposed to a high temperature of 34 C for 24 hours and it was determined that plasma corticosterone values were greatly increased (34). Young growing rats had plasma corticosterone levels almost twice as high as mature rats after 24-hour heat exposure. At three, six, or nine weeks of exposure, there was no significant difference between rats exposed to 34 C and their controls maintained at a 28 C environment. This was apparently due to some manner of acclimatization after the initial exposure to heat.

Thompson et al. (76) found that Holstein heifers maintained under controlled ambient conditions with a hot temperature ranging from 75 to 95 F (24 to 35 C) exhibited a mean 17-hydroxycorticosterone level of 4.5 micrograms percent. This was a highly significant increase over a mean of 1.8 micrograms percent for heifers kept under cool

conditions of 40 to 65 F (4.4 to 18 C).

Holstein steers exposed to a heat stress of 42 C and 60% relative humidity for 240 minutes displayed mean glucocorticoid levels showing a significant heat effect at 240 minutes (12). The glucocorticoid levels increased from 35 ng/l at -20 minutes to 46 ng/l at 240 minutes. Mean glucocorticoid levels on the plasma of these same animals ranged from 35 ng/l at 240 minutes when not subjected to heat stress. Twenty minutes after the discontinuance of heat stress, plasma glucocorticoids had returned to 34 ng/l. This points out the rapidity with which circulating glucocorticoid levels can change in response to an environmental change.

However, Lee (35) found that lactating Holstein cows exposed to direct sunlight during the Louisiana summer season showed no elevation in plasma corticosterone when compared to animals maintained under shade. Bergman (4) observed a significant depression in both plasma cortisol levels and cortisol secretion rates in mature cows maintained at a constant temperature of 29 C.

While the literature reviewed does show agreement on many aspects of adrenal cortex physiology, there are areas of uncertainty which exist. Apparently, not only the species of animal must be taken into account, but such factors as age, sex, lactation and reproductive status, and

any other variable which can affect homeostasis. In regard to heat stress, it is yet to be ascertained exactly what constitutes such a condition in a given degree and the effects it will have on a given organism.

III. EXPERIMENTAL METHODS

A. General Outline

1. Assignment of Animals

Lactating Holstein cows from the Louisiana State University dairy herd were used in this experiment. All animals showed freedom from any evidence of mastitis and other clinical diseases 21 days prior to selection. The animals were partitioned into five categories of reproductive status as outlined in Table 2.

Table 2. Reproductive status classifications of the experimental animals.

Reproductive Status	Description
1	Pregnant 1-90 days
2	Pregnant 91-180 days
3	Open, normal breeder
4	Open, anestrus
5	Open, cycling, 4+ services/conception

The animals were stratified according to stage and number of lactations and selected at random each month for

sample collection. Eighteen to 24 animals with an average of 22 animals per month were utilized for data collection.

This manner of selection permitted, but did not dictate, the collection of data from an individual animal more than once. Consequently, data were collected from individual cows from one to 10 times. A total of 264 cow-months were used in this study.

2. Feeding and Management

All animals were offered 100% of the National Research Council (43) estimated net energy requirements in their dietary regime. The ration consisted of Number 1 leafy alfalfa hay, good corn silage, 16% crude protein concentrate pellet mixture, and 2% dicalcium phosphate. The roughage consisted of 75% corn silage and 25% alfalfa hay. Both silage and concentrates were offered three times daily and hay twice daily. Trace mineralized salt in block form and water were available ad libitum.

Standard management and milking procedures established for the Louisiana State University dairy herd were employed throughout the experiment. All animals were milked twice daily. The interval between AM and PM milking was 11 hours and between PM and AM milking 13 hours.

3. Duration of the Experiment

The animals were on experiment for one calendar year, from January through December, 1969. It has been

established (52) that the yearly Louisiana temperature usually ranges from a minimum of approximately -4 C in the cool season to a maximum of approximately 38 C in the hot, humid season. Because of this wide temperature variation, the calendar year was divided into three periods to correspond with the cool, intermediate, and hot Louisiana ambient seasons. Temperature-season 1 consisted of the cool winter months of October, November, December, and January. Temperature-season 2 was comprised of the four intermediate or optimum months of February, March, April, and May. Temperature-season 3 was representative of the hot season and consisted of June, July, August, and September.

The method of partitioning the year into temperature-seasons resulted in temperature-season 1 being comprised of three consecutive months and one non-consecutive month. The other two temperature-seasons each included four consecutive months. The data from temperature-seasons 1, 2, and 3 consisted of 90, 87, and 87 cow-months, respectively.

B. Collection of Data

1. Blood Collection

Blood samples were obtained from individual animals on the fifteenth day of each month $\pm$four days. Blood samples were taken by jugular vein puncture between 8 AM and 9 AM. Bleeding at the same hour each month ruled out

the possibility of daily diurnal effects confounding the analytical results. Restraint, venipuncture, and blood collection were accomplished as rapidly as possible with the procedure normally lasting two to three minutes. Samples were rejected from animals which displayed excessive excitement or in which the collection time was prolonged which might tend to cause a disproportionate change in plasma corticosteroid level. Blood was received directly into two 40 ml collection tubes. One collection tube contained three drops of an anticoagulant (dipotassium ethylene diamine tetra-acetate) and this sample was used in the analysis requiring whole blood with the remainder being centrifuged (886 x G for 20 minutes) and the plasma withdrawn, frozen, and stored under nitrogen at -4 C for subsequent analyses.

A portion of the second sample of whole blood was used immediately for hematocrit determination. Serum samples were obtained from the remaining portion by means of centrifugation (886 x G for 20 minutes) of the coagulated whole blood. The serum was withdrawn, frozen, and stored for later analyses.

4. Cortisol Determination

The cortisol concentrations of the plasma samples were determined by the competitive protein-binding method of Murphy (42) as modified by Stott (73). This method

entails removing the plasma-binding proteins to preclude their acting in the same way as the assay protein, that is, corticosteroid-binding globulin (CBG). Next, free corticoid is mixed with an aliquot of CBG and a radioactive tracer. Tritum was used as the tracer in this work. Murphy (42) reported a 100-fold increase in sensitivity by using tritiated steroids as opposed to ^{14}C-labeled steroids. The conditions employed are such that the CBG is saturated with tracer steroid and a dynamic equilibrium exists between the CBG-bound steroid and the unbound steroid. The sample steroid displaces a portion of the tracer and the CBG-bound tracer decreases in a proportionate manner. The protein-bound and unbound fractions are separated and the tracer distribution determined and quantitated (42).

In this experiment, the CBG was prepared using serum from a dog receiving diethylstilbestrol (1mg/day) to reduce steroid variability (73). Dog serum is reported to have a very high affinity for cortisol (82).

The method used for CBG preparation follows:

(1) Placed 0.25 mci of corticosterone -1, 2 -H^3 into a 25 ml volumetric flask and brought to volume with absolute ethanol. Stored in individual glass vials (0.5 ml/vial) in freezer;

(2) Placed 5mci corticosterone -1, 2 -H^3 (1 vial) in a 100 ml volumetric flask;

(3) Added 75 ml PO_4 buffer (0.1 molar KH_2PO_4 and K_2HPO_4 in distilled water);

(4) Added 2.5 ml dog serum and mixed well and;

(5) Brought to volume with PO_4 buffer and stored in dark-glass bottle in refrigerator.

Scintillation fluid for cortisol analysis was prepared as follows:

(1) Placed 0.3 gm POPOP (2, 2' paraphentlene bis 5- phenyloxazole) and 5.0 gm PPO (2,5-diphenyloxazole) in a one liter volumetric flask;

(2) Brought to volume with toluene and mixed well and;

(3) Added 100 ml Biosolve (Beckman formula BBS-3), mixed and stored in a dark-glass bottle.

The competitive protein-binding assay for cortisol concentration in the plasma samples was accomplished as follows:

(1) Pippeted 50 lambda of plasma into a 12 ml conical centrifuge tube;

(2) Added 500 lambda ethanol and mixed for 30

seconds on a Vortex mixer;

(3) Centrifuged (886 x G for 15 minutes) to separate layers;

(4) Removed ethanol with a disposable pipet into a 15 x 85 mm culture tube;

(5) Repeated ethanol extraction;

(6) Evaporated to dryness, under nitrogen, in a water bath at 40-50 C;

(7) Added one ml cold CBG to each tube, shook 10 seconds and incubated for five minutes in a 45 C water bath;

(8) Cooled in ice bath for ten minutes;

(9) Added approximately 40 mg Florisil to the tube and shook for 30 seconds;

(10) Paused two minutes and removed 500 lambda CBG to counting vial containing 15 ml scintillation fluid and;

(11) Prepared standards containing 0, 1/2, 1, 2, 3, and 4 ng corticosterone -1, 2-H^3 in a vial containing 15 ml scintillation fluid.

Samples and standards were counted with a liquid scintillation counter (Beckman LS-250 Liquid Scintillation System). A vial containing only scintillation fluid was counted at each counting period and this value was subtracted from the total count per minute (CPM) to obtain

corrected net counts per minute. For convenience, the data were expressed as minutes per 25,000 counts.

Standard curves were constructed on a semi-logarithmic graph and plotted as minutes per 25,000 counts versus concentration (ng). The sample concentrations were read directly from the standard curve. As each sample contained 50 lambda serum, the concentration of the sample, as determined from the standard curve, was multiplied by 20 to express sample concentration as ng cortisol per ml plasma.

To reduce variability associated with the CBG, a new standard curve was plotted each time new CBG was prepared.

3. Hematocrit Determination

Whole blood was introduced into Wintrobe hematocrit tubes and centrifuged at 1121 x G for 30 minutes. Packed red cell volumes were read directly from the tubes.

4. Hemoglobin and Oxyhemoglobin

Blood hemoglobin levels were determined by the method of Cannan (11). This method included mixing 0.02 ml of whole blood with 5.0 ml of Drabkin's Reagent in a Fisher hemophotometer tube. The mixture was allowed to stand ten minutes and then the hemoglobin concentration (grams percent) was read on a hemophotometer (Fisher Hemophotometer). Drabkin's reagent was prepared by introducing

1.0 g sodium bicarbonate, 50 mg potassium cyanide, and 200 mg potassium ferric-cyanide into a liter volumetric flask and bringing to volume with distilled water.

Oxyhemoglobin concentrations were determined by the method of Collier (13). This method involved mixing 0.02 ml of whole blood with 50 ml of 0.4% NH_4OH in a Fisher hemophotometer tube. The mixture was allowed to stand ten minutes and then read on a hemophotometer.

5. Blood Cell Profile

Red blood cell and total leukocyte counts were made on anti-coagulated whole blood immediately after collection. An A O Bright-Line hemacytometer was employed for all counts and Hayem's fluid (0.5 g mercury bichloride, 1.0 g sodium chlorine, 5.0 g sodium sulfate, and 200 ml distilled water) was used for red blood cell dilution. The dilution fluid for total leukocytes was 10 mg crystal violet in 1.0% glacial acetic acid.

Blood smears were made at the time of bleeding and stained with Wrights-Giesma stain (Fisher Scientific Co.) and later counted for differential leukocyte profile. The differential count included eosinophils, neutrophils, and lymphocytes.

6. Blood Protein Determinations

A modification of the method of Weichselbaum (84) was employed for the determination of total serum protein

concentrations. In this procedure, 0.1 ml serum was added to 6.0 ml Stable Buiret Reagent (Hycel) and incubated in a water bath at 30 C for 30 minutes. The absorbance of the solution was then read on a spectrophotometer (Hitachi Perkin-Elmer-139) at a wavelength of 540 mu. Protein concentration was obtained by comparing the optical density to a standard curve of known concentrations.

Serum protein fractions were determined by the method of Hycel, Inc. (30) which utilized differential precipitation and turbidimetric analysis. This method involved mixing 0.5 ml serum and 4.5 ml serum diluent (Hycel) and allowing it to stand five minutes. Then, 0.6 ml of the mixture was transferred to a mixture of 6.0 ml distilled water and a pre-prepared commercial solution (Hycel) and mixed. After 15 minutes, the optical density was read against a water blank on a spectrophotometer at a 525 mu wavelength. One of five commercial solutions was used for the determination of a respective protein fraction. By knowing the total protein, it was possible graphically to quantitate the fractions in the following manner.

Absorbance (A.)1 = total protein

A.1 - A.2 = albumin

A.2 = globulins

A.2 - A.3 = alpha 1 globulins

A.3 - A.4 = alpha 2 globulins

A.4 - A.5 = beta globulins

A.5 = gamma globulins

7. Milk Production

Milk production was recorded daily and a representative bucket sample from a PM and an AM milking was taken once monthly for fat (lipid) determination. Milk fat percent was determined by a milko-tester (MKll) (A/S N. Foss Electric). Milk production was expressed on the basis of 4.0 percent fat corrected milk (FCM) as calculated by the formula 0.4 (total milk) + 15 (total fat) = FCM.

8. Rectal Temperature and Respiration Rate

Rectal temperatures and respiration rates were recorded immediately prior to bleeding. A Thermistor thermometer with a rectal probe was used to measure rectal temperature and the reading was recorded to the nearest one-tenth degree centigrade. Respiration rates were determined by counting flank movements for one minute.

C. Statistical Analyses

The data were statistically analyzed according to the methods of Steele and Torrie (71). The data were subjected to analysis of variance to partition out total

season, reproductive, and lactation effects. Because of the inequality of subclass numbers, least squares procedures were used to determine any significant differences attributal to these effects. Simple correlations between certain variables were determined. The data were analyzed at the Louisiana State University Computer Research Center.

IV. RESULTS AND DISCUSSION

The data for this study were collected from lactating Holstein cows over a period of one calendar year. The calendar year was divided into three temperature-seasons to represent the hot, intermediate, and cool Louisiana climatic seasons. The study was comprised of a total of 264 cow-months, with the individual animals appearing in the data from one to 10 times.

This investigation was conducted to study the effects of ambient conditions on certain physiological responses in the lactating bovine over a relatively long time period. Additionally, the data were collected in a manner so as to enable the determination of the effects of reproductive status, lactation number, and age on the physiological responses. Measurements made included circulating plasma cortisol level, hematocrit, red blood cell numbers and leukocyte profile, hemoglobin, oxyhemoglobin, blood protein fractions, milk production, rectal temperature, and respiration rate.

A. Climatic Conditions

Measures of climatic conditions for the duration of the experiment included maximum and minimum temperature,

maximum and minimum humidity, dew point, and temperature humidity index (THI) for 3, 7, and 14 days prior to monthly sample collection. THI was constructed from the formula THI = .72 /dry bulb temperature (C)+ wet bulb temperature (C)+ 40.67, as described by Maust et al. (40).

A summary of the mean climatic measurements for the three temperature-seasons is presented in Table 3. These data show that the maximum relative humidity remained the same for the cool and intermediate temperature-seasons (97.5%) with an increase to 99.9% for the hot temperature season. Minimum relative humidity declined from a high of 55.5% in the cool temperature-season to a low of 40.0% in the hot temperature-season. All other climatic factors measured increased from the cool through the hot temperature-seasons. Maximum temperature, minimum temperature, dew point, and THI showed overall increases of 64.0, 48.5, 75.3, and 82.2%, respectively, for the three temperature-seasons.

B. Plasma Cortisol

The highest levels of circulating plasma cortisol were found in the cooler months which comprised temperature-season 1 (Table 4). The mean for the plasma cortisol in this temperature-season was 42.4 ng/ml. The values fell to a mean of 36.3 ng/ml into the intermediate months of temperature-season 2. As the ambient season became warmer, the

Table 3. Mean climatic measurements for the three temperature-seasons at 3, 7, and 14 days prior to sample collection.

Temperature-Season		Temperature		Relative Humidity		Dew Point	THI
		Max	Min	Max	Min		
		- -(C)- -		- - (%) - -		(C)	
1-Cool day,	-3	20.3	10.4	100.0	61.2	54.5	65.9
	-7	21.7	10.3	100.0	58.2	57.0	68.0
	-14	22.5	9.9	92.5	47.2	54.0	70.7
Mean		21.5	9.9	97.5	55.5	55.2	68.2
2-Intermediate day,	-3	22.9	11.4	96.4	48.0	61.0	69.4
	-7	21.8	9.6	98.0	38.5	57.8	68.3
	-14	20.8	11.1	98.0	48.7	60.5	72.0
Mean		21.8	10.7	97.5	45.1	59.8	69.9
3-Hot day,	-3	34.0	20.3	100.0	42.0	71.5	83.1
	-7	34.2	20.5	100.0	39.2	74.5	83.9
	-14	32.7	20.3	99.8	38.8	73.8	82.1
Mean		33.6	20.4	99.9	40.0	73.3	83.0

plasma cortisol levels further declined to a mean of 22.8 ng/ml in the temperature-season 3. The response of plasma cortisol level to temperature-season is graphically illustrated in Figure 5.

Analysis of variance revealed that this response to temperature-season was significant ($P<0.01$). It was further revealed that this response was linear ($P<0.01$).

Table 4. Effect of temperature-season on plasma cortisol level.

Temperature-season	Cortisol (ng/ml)
1-Cool	42.3
2-Intermediate	36.3
3-Hot	22.8

Table 5 shows that a significant ($P<0.01$) negative correlation coefficients exist between the circulating plasma cortisol levels and the ambient climatic measurements of maximum and minimum temperatures, dew point, and THI at 3, 7, and 14 days prior to collection. These negative correlations substantiate the validity of the season effect by showing an inverse relationship between cortisol level and those factors normally associated with ambient heat stress. The correlation coefficients presented in Table 5 show by both sign and magnitude, that the influence

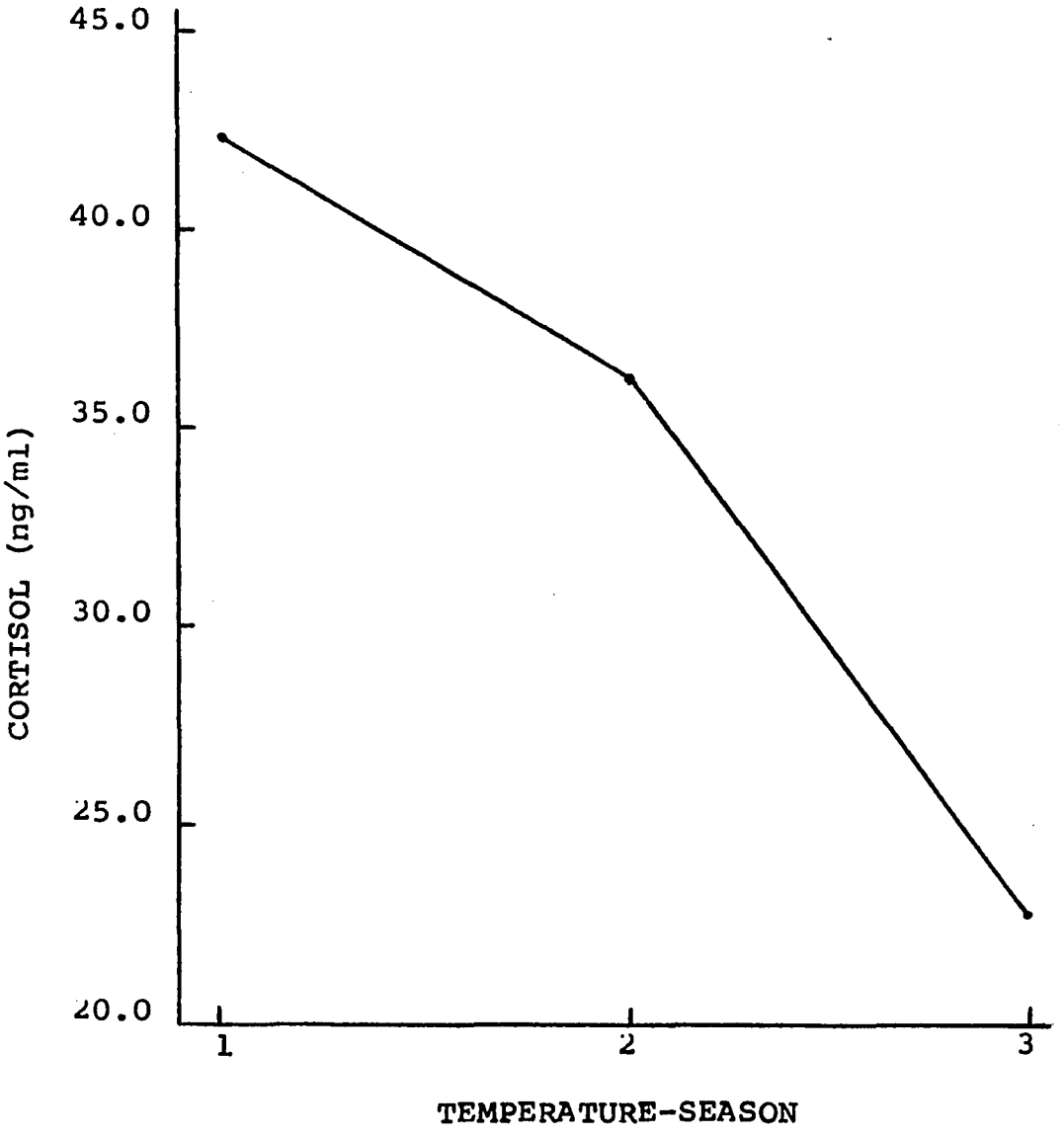


Fig. 5. Relationship between temperature-season and plasma cortisol levels.

Table 5. Correlation coefficients between ambient climatic measurements and plasma cortisol levels 3, 7, and 14 days prior to sample collection.

Ambient factors	Cortisol
	(r)
Max T, -3	-0.23**
Min T, -3	-0.29**
Max H, -3	-0.01
Min H, -3	-0.02
D.P., -3	-0.18**
THI, -3	-0.23**
Max T, -7	-0.23**
Min T, -7	-0.34**
Max H, -7	-0.19**
Min H, -7	-0.06
D.P., -7	-0.29**
THI, -7	-0.25**
Max T, -14	-0.22**
Min T, -14	-0.28**
Max H, -14	-0.36**
Min H, -14	-0.12
D.P., -14	-0.26**
THI, -14	-0.24**

** ($P<0.01$).

exerted on adrenal cortex function at 14 days prior to sample collection is essentially the same as the influence exerted at seven and three days prior to sample collection.

It has been generally accepted that heat stress causes an increased ACTH secretion which, in turn, produces an increase in adrenal-cortical activity. Workers (12, 66) have reported an increase in circulating corticoid levels in response to heat stress in the bovine. However, these experiments were usually of a short duration and involved a sudden exposure to heat stress. A search of the literature revealed that this research provides the only data regarding bovine adrenal cortical response to ambient conditions throughout a 12-month period of time.

In this study, the animals were apparently able to adjust physiologically so as to compensate for elevated heat loads. Thus, glucocorticoid secretion, namely cortisol, may not have been governed by increased ACTH secretion in response to stress. Glucocorticoids have a metabolic effect of increasing heat production (77). It may be postulated that when time is allowed for acclimation, the calorogenic effect of the glucocorticoids becomes of primary concern. This would account for the lower plasma cortisol levels being found in the hotter months as an attempt to reduce metabolic heat loads. In the cooler months, when more metabolic heat production could be

tolerated, proportionately higher cortisol levels were found. These findings are in agreement with those of Bergman (4) who found a depression in hydrocortisone levels in mature cows exposed to continuous high ambient temperatures.

Workers (1) have reported an increase in corticoid level in the pregnant bovine one to four days prior to parturition. However, this study was carried out during the lactation period and was not designed to detect any changes immediately prior to parturition. Stott and Thomas (75) have reported that heifers conceiving on the first service displayed higher corticoid levels than those with breeding anomalies. The data in Table 6 do not confirm this in that open, normal breeders had plasma cortisol levels of nearly the same magnitude as problem breeders (32.6 ng/ml versus 30.8 ng/ml). However, in the work where differences were reported, animals with higher corticoid levels were also subjected to a stress of dietary deficiency which may have confounded the results. The animals in this study were not subjected to any such dietary deficiencies.

Research by Paterson and Harrison (48) revealed no changes in the cortisol secretion rate during pregnancy in sheep. Shaw et al. (61) in 1960, reported that pregnancy had no influence on plasma glucocorticoid levels in

the bovine. However, it is probable that the methods used at that time lacked the accuracy and sensitivity of the competitive protein-binding method employed in this study.

The data presented in Table 6 show that animals in the first 90 days of pregnancy possessed the highest level of plasma cortisol (47.5 ng/ml) in regard to reproductive status. Animals pregnant longer than 90 days displayed plasma cortisol level (29.1 ng/ml) of essentially the same magnitude as non-pregnant animals. Therefore, it is indicated that the requirements for circulating cortisol in the first trimester of pregnancy is higher than at any other time. This implies a unique feature of bovine physiology which is apparently not shared by all mammalian species.

Table 6. Relationship between reproductive status and plasma cortisol level.

Reproductive status	Cortisol
	(ng/ml)
1-Pregnant 1-90 days	47.5
2-Pregnant 91-180 days	29.1
3-Open, normal breeder	32.6
4-Open, anestrus	33.3
5-Open, regular cycle, 4+ services/conception	30.8

An increase in circulating glucocorticoids may be necessary for the bovine to adapt to the state of pregnancy, or the increase may be necessary to maintain a balance with placental hormones in the first trimester of pregnancy.

A significant ($P<0.01$) negative correlation coefficient (-0.32) was found to exist between animal age and circulating cortisol level. Similarly, significant ($P<0.01$) negative correlation coefficient of -0.31 between lactation number and plasma cortisol level was obtained. It is to be noted that lactation number would normally be a reflection of animal age.

C. Hematocrit, Red Blood Cells, Hemoglobin and Oxyhemoglobin

Statistical analysis revealed a significant ($P<0.05$) linear effect of season on hematocrit. Figure 6 shows that although there was little difference in the hematocrit values determined for the cool and intermediate seasons (35.9% and 36.0%), there was a marked depression during the hot season (34.0%).

Examination of Table 7 reveals that correlation coefficients between ambient factors and the values for hematocrit, red blood cells, hemoglobin, and oxyhemoglobin are predominantly negative. This indicates that as climatic measurements indicative of heat stress increase, there

is a resulting decrease in these blood constituents.

Furthermore, it may be assumed that the seasonal effect on hematocrit can be partly attributed to decreases in red blood cells during periods indicative of environmental heat stress. The negative correlations associated with hemoglobin and oxyhemoglobin (Table 7) tend to support this supposition. Other workers (44, 53) verify a depression in hematocrit and red blood cells in cattle subjected to elevated environmental temperatures.

The lowered hematocrits under higher temperatures may be due to a physiological adjustment to heat stress whereby metabolism is reduced in order to lessen metabolic heat load. Such an adjustment would tend to decrease cellular oxygen requirements. Consequently, a reduction in red blood cells and hematocrit would be associated with a decrease in oxygen transport.

According to the analysis of variance, a significant ($P<0.05$) effect of reproductive status on red blood cell numbers existed. A plot of these data is presented in Figure 7 and the effect is quadratic. Animals pregnant 1-90 days showed the highest level of circulating red blood cells (775.6 cells/cmm). Animals pregnant 91-180 days, non-pregnant normal breeders, and cycling problem breeders displayed red blood cell values of 725.0, 724.0, and 742.0 cells/cmm, respectively. The lowest number

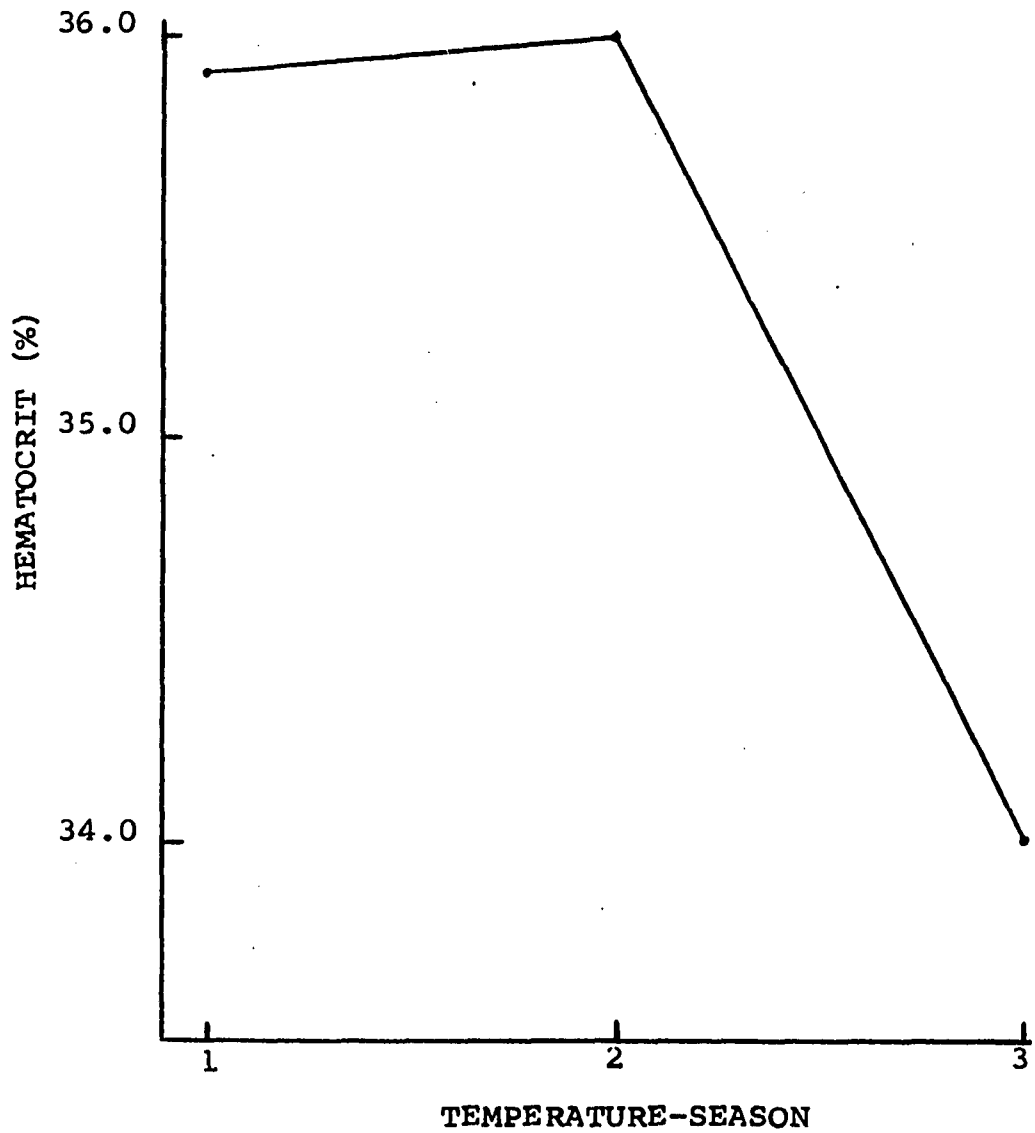


Fig. 6. Relationship between temperature-season and percent hematocrit.

Table 7. Correlation coefficients between ambient climatic conditions and hematocrit, red blood cells, hemoglobin, and oxyhemoglobin values.

Ambient factors	Hematocrit	RBC	Hb	O_2Hb
	- - - - - - - - - - (r) - - - - - - - - - -			
Max T, -3	-0.18**	-0.09	-0.11	-0.16*
Min T, -3	-0.20	-0.15*	-0.17**	-0.09
Max H, -3	-0.02	-0.05	-0.03	-0.11
Min H, -3	0.40**	-0.01	-0.08	0.12*
D.P., -3	0.10	-0.19**	-0.10	0.10
THI, -3	-0.12	-0.12*	-0.11	-01.0
Max T, -7	-0.21**	-0.19**	-0.16**	-0.25**
Min T, -7	-0.15*	-0.22**	-0.20**	-0.21**
Max H, -7	-0.02	-0.09	-0.15*	-0.15*
Min H, -7	0.12**	-0.06	-0.12*	-0.04
D.P., -7	-0.10	-0.28**	-0.20**	-0.14*
THI, -7	-0.18**	-0.22**	-0.17**	-0.22**
Max T, -14	-0.23**	-0.23**	-0.20**	-0.26**
Min T, -14	-0.18**	-0.22**	-0.21**	-0.23**
Max H, -14	-0.31**	-0.16**	0.03	-0.15*
Min H, -14	0.13*	-0.05	-0.07	-0.02
D.P., -14	-0.16**	-0.20**	-0.20**	-0.19**

(continued)

Table 7 (continued). Correlation coefficients between ambient climatic conditions and hematocrit, red blood cells, hemoglobin, and oxyhemoglobin values.

Ambient factors	Hematocrit	RBC	Hb	O_2Hb
THI, -14	-0.22**	-0.23**	-0.21**	-0.25**

* (P<0.05).
** (P<0.01).
RBC = Red blood cells.
Hb = Hemoglobin
O_2Hb = Oxyhemoglobin

of circulating red blood cells (689.7 cells/cmm) were found in the non-pregnant, anestrus animals.

These findings may be interpreted to indicate that early pregnancy promotes cellular metabolism and increases the demand for oxygen consumption. Conversely, it appears that a reduction in cellular metabolism and oxygen consumption is associated with the non-pregnant, anestrus state.

Although not statistically significant, hematocrit, hemoglobin, and oxyhemoglobin tended to follow the same pattern in regard to reproductive status. The data in Table 8 show that anestrus animals had the lowest values for hematocrit (33.8%), hemoglobin (9.9 g/100 ml), and oxyhemoglobin (9.3 g/100 ml).

Table 8. Relationship between reproductive status and hematocrit, hemoglobin, and oxyhemoglobin levels.

Reproductive status	Hematocrit	Hb	O_2Hb
	(%)	(g/100ml)	(g/100ml)
1-Pregnant 1-90 days	38.2	10.7	10.3
2-Pregnant 91-180 days	38.0	11.0	10.9
3-Open, normal breeder	35.6	10.3	9.7
4-Open, anestrus	33.8	9.9	9.3
5-Open, regular cycle, 4+ services/conception	34.7	10.0	9.4

Hb = Hemoglobin
O_2Hb = Oxyhemoglobin

D. Leukocyte Profile

The data in Table 9 show that the greatest number of total circulating leukocytes was found in the cool temperature-season. Examination of Figure 8 reveals a decrease in total leukocytes for the intermediate temperature-season, and then an elevation during the hot temperature-season. Further examination of Figure 8 shows this same pattern to exist for the number of circulating lymphocytes and neutrophils. However, while the increase in circulating lymphocytes from the intermediate to hot temperature-season is slight (5.3%), the increase in neutrophils in this same time period is much more pronounced (20.1%).

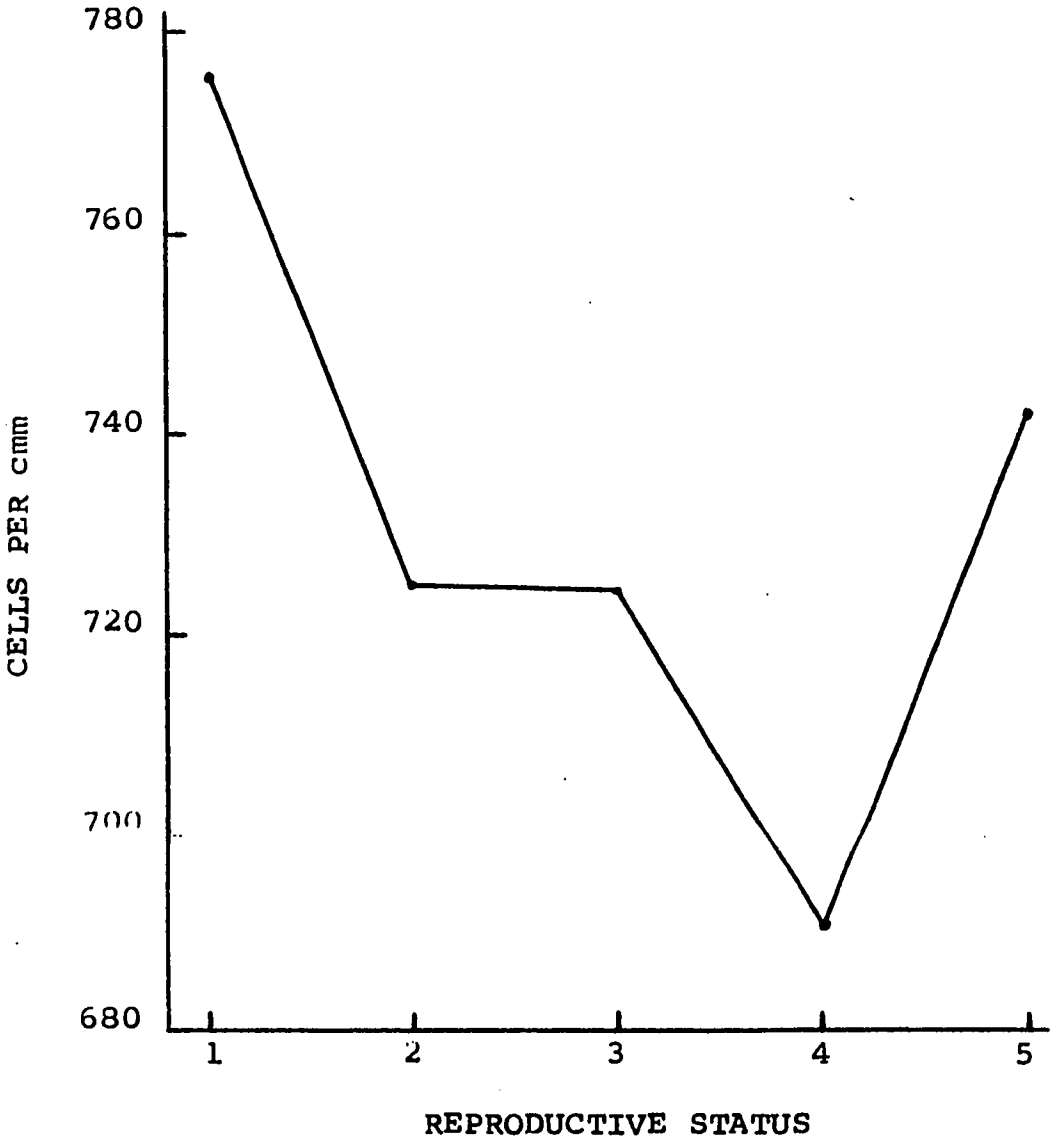


Fig. 7. Relationship between reproductive status and circulating red blood cells.

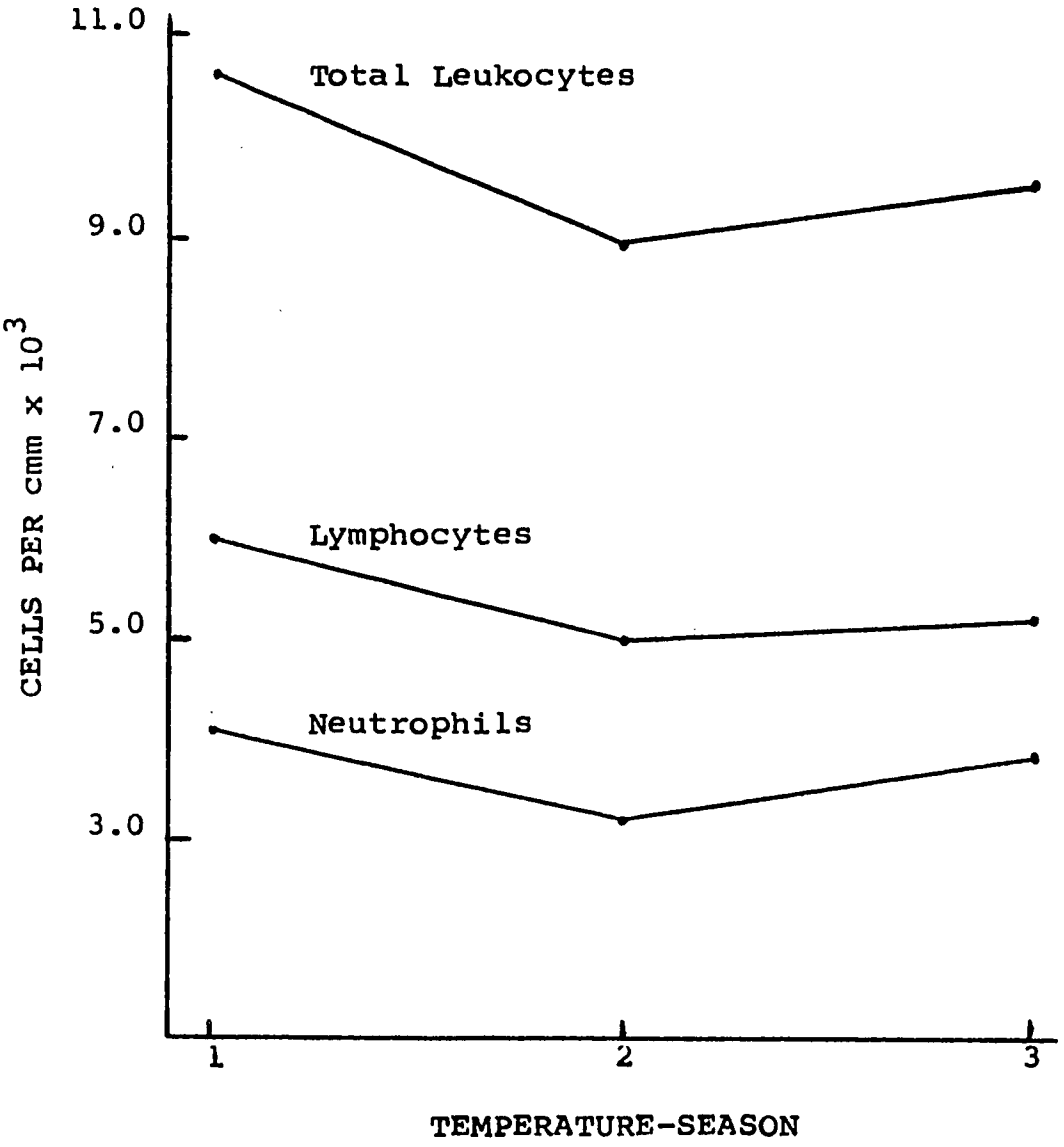


Fig. 8. Relationship between temperature-season and circulating total leukocytes, lymphocytes, and neutrophils.

Thus, it is demonstrated that most of the leukocytosis affected by increasing ambient temperature can be accounted for by a change in the number of circulating neutrophils. Wegner and Stott (83) reported a similar lymphocyte and neutrophil pattern in response to ACTH injection.

There was no change in the number of circulating eosinophils between the cool and intermediate temperature-seasons. However, there was a 26.7% increase in the number of circulating eosinophils from the intermediate to the hot temperature-season (Figure 9). Conversely, other workers (55, 60, 69, 83) reported an eosinopenia in response to ACTH injection to stimulate physiological stress. However, the findings presented here are in agreement with research in which cattle were subjected to the stress of high ambient temperatures (32, 53). This seems to indicate that a sudden stress situation, as from ACTH injection, results in an eosinopenia, but a stress imposed in a more gradual manner yields an eosinophilia.

The leukocyte profile in regard to reproductive status is illustrated in Figure 10. Animals of reproductive status 3 (open, normal breeder) displayed a greater number of circulating total leukocytes, lymphocytes, and neutrophils than did animals of reproductive status 1 (pregnant 1-90 days), or 2 (pregnant 91-180 days), or 4 (open, anestrus). Furthermore, the number of circulating

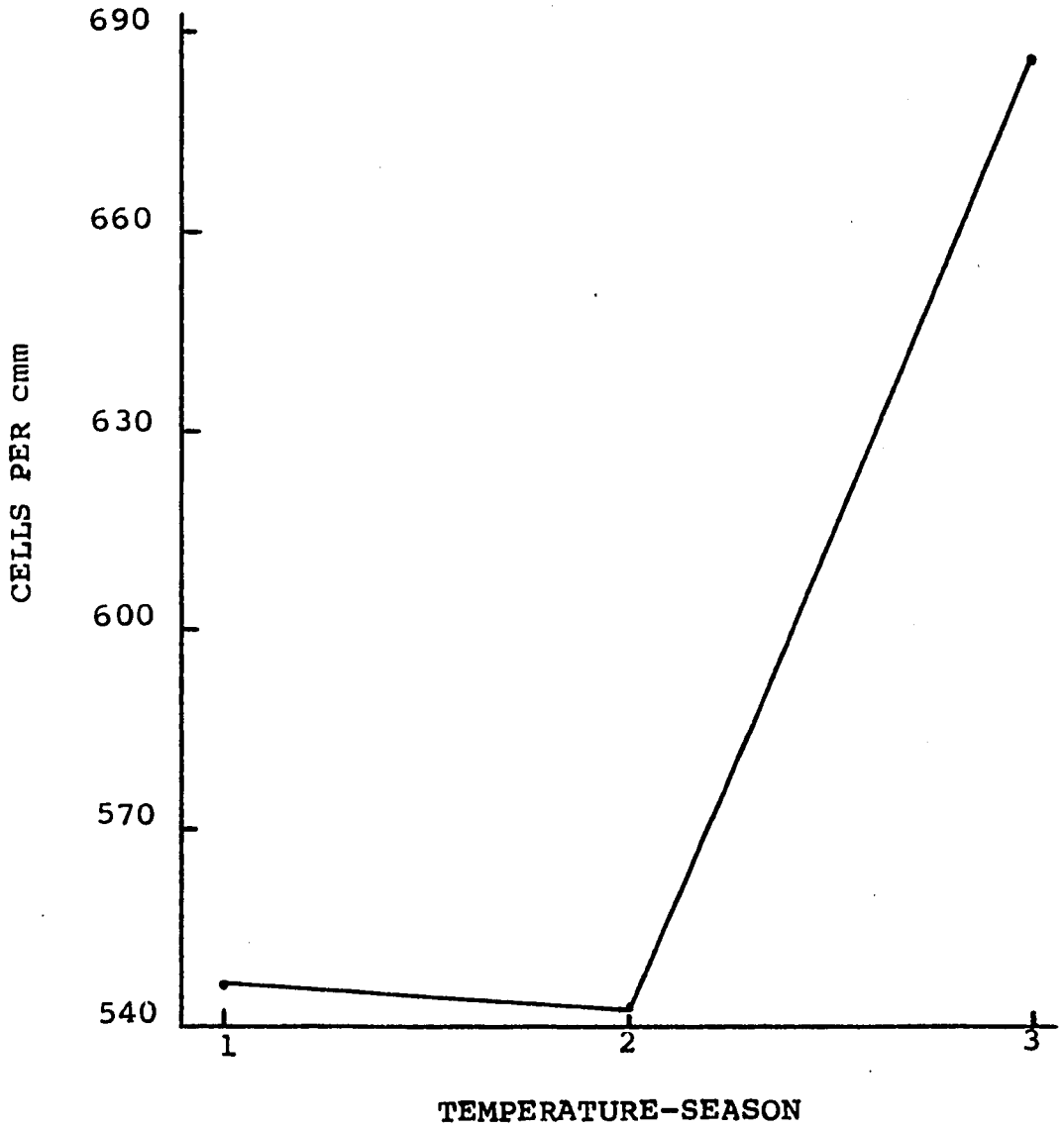


Fig. 9. Relationship between temperature-season and circulating eosinophils.

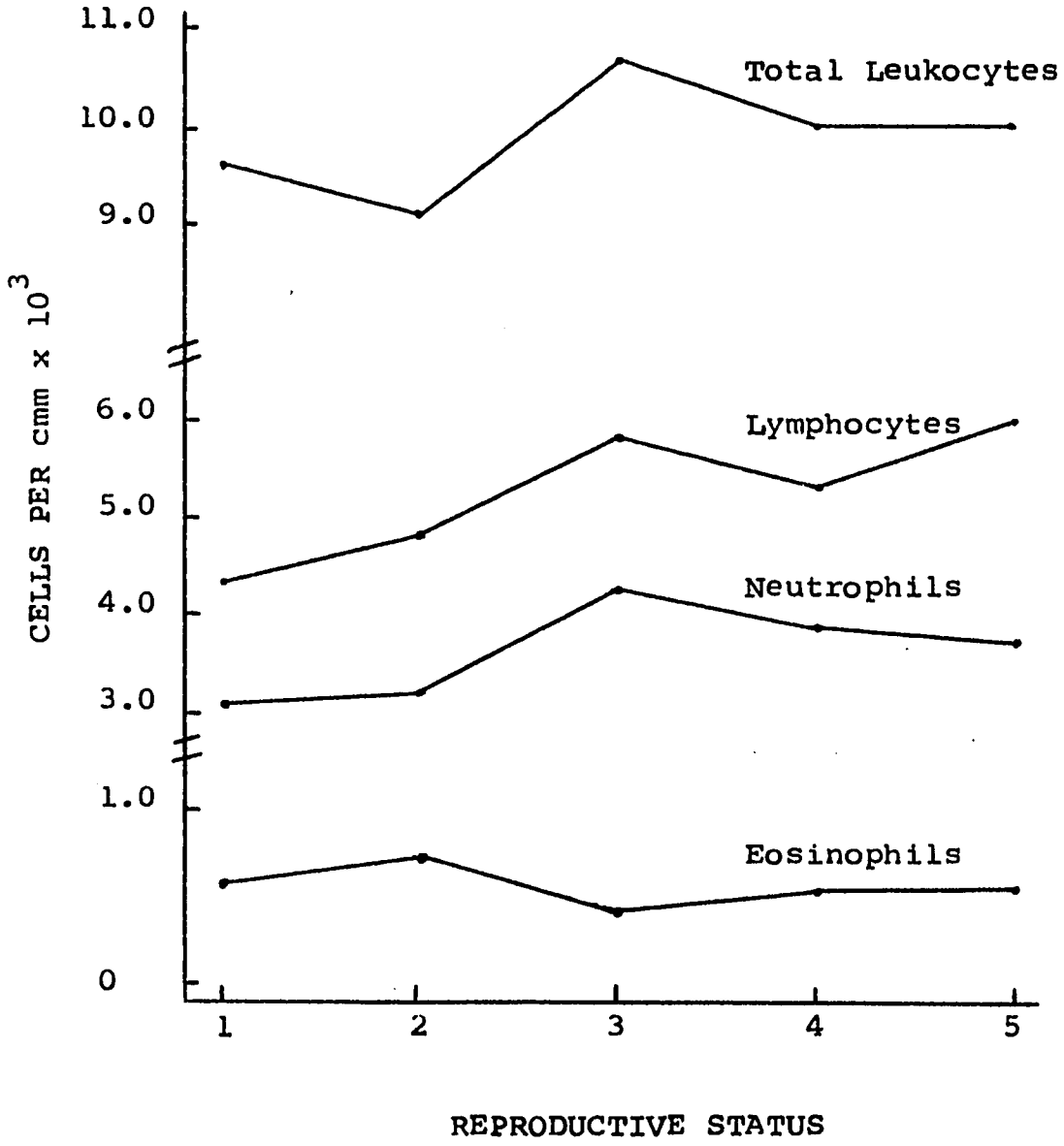


Fig. 10. Relationship between reproductive status and leukocyte profile.

total leukocytes and neutrophils were higher for reproductive status 3 than for reproductive status 5 (open, regular cycle, 4+ services per conception). However, the greatest number of circulating lymphocytes was found for reproductive status 5.

Table 9. Temperature-season effects on leukocyte counts.

Temperature-season	Total leukocytes	Neutrophils	Lymphocytes	Eosinophils
	------(cells/cmm)--------			
1-Cool	10,728	4,087	6,012	546
2-Intermediate	8,977	3,178	4,966	542
3-Hot	9,539	3,816	5,231	686

E. Blood Proteins

Table 10 presents correlation coefficients between measurements of ambient conditions and the total serum protein, albumin, and globulin. With the exception of minimum humidities, all of the correlation coefficients of total serum protein are positive and significant ($P<0.01$). This indicates that warmer seasonal conditions promote an associated increase in total serum protein. These ambient measures exhibited negative correlation coefficients with serum albumin and positive coefficients with serum globulin.

Table 10. Correlation coefficients between ambient climatic measurements and serum total protein, albumin, and globulin values.

Ambient factors	Total protein	Albumin	Globulin
	- - - - - - - - - - - - (r) - - - - - - - - - - - -		
Max T, -3	0.21**	-0.20**	0.22**
Min T, -3	0.18**	-0.15*	0.17**
Max H, -3	0.19**	-0.14*	0.12*
Min H, -3	-0.22**	0.16*	-0.17**
D.P., -3	0.15**	-0.10	0.11
THI, -3	0.21**	-0.18**	0.21**
Max T, -7	0.23**	-0.23**	0.25**
Min T, -7	0.28**	-0.24**	0.25**
Max H, -7	0.19**	-0.15*	0.13*
Min H, -7	-0.08	0.02	-0.06
D.P., -7	0.25**	-0.21**	-0.22**
THI, -7	0.24**	-0.23**	0.25**
Max T, -14	0.21**	-0.22**	0.23**
Min T, -14	0.24**	-0.22**	0.23**
Max H, -14	0.63**	-0.37**	0.33**
Min H, -14	0.03	0.07	-0.07
D.P., -14	0.26**	-0.19**	0.21**
THI, -14	0.22**	-0.22**	0.23**

* ($P<0.05$).
** ($P<0.01$).

A plot of the total serum protein values (Figure 11) shows an increase from 7.54 mg% in the cool temperature-season to 7.75 and 7.97 mg% in the intermediate and hot temperature-seasons, respectively.

Further examination of Figure 11 reveals that blood serum globulins underwent a slight decrease (4.99 to 4.89 mg%) from the cool to the intermediate temperature-season and then an increase to 5.47 mg% for the hot temperature-season. An opposite pattern was found for blood serum albumins. Albumins underwent a slight increase (2.63 to 2.86 mg%) from the cool to the intermediate temperature-season, and then decreased to 2.50 mg% during the hot temperature-season.

Plots of $alpha_1$, $alpha_2$, beta, and gamma globulins are presented in Figure 12. $Alpha_1$ globulin values went from a low of 0.30 mg% in the cool temperature-season to a high of 0.44 mg% in the intermediate temperature-season. This fraction then declined to 0.31 mg% during the hot temperature-season. Conversely, the $alpha_2$ fraction fell from a high of 0.58 mg% in the cool temperature-season to a low of 0.42 mg% in the intermediate temperature-season. An elevation of 0.47 mg% was observed for the hot temperature-season.

The beta globulin fraction increased from a low of 1.55 mg% in the cool temperature-season to a high of

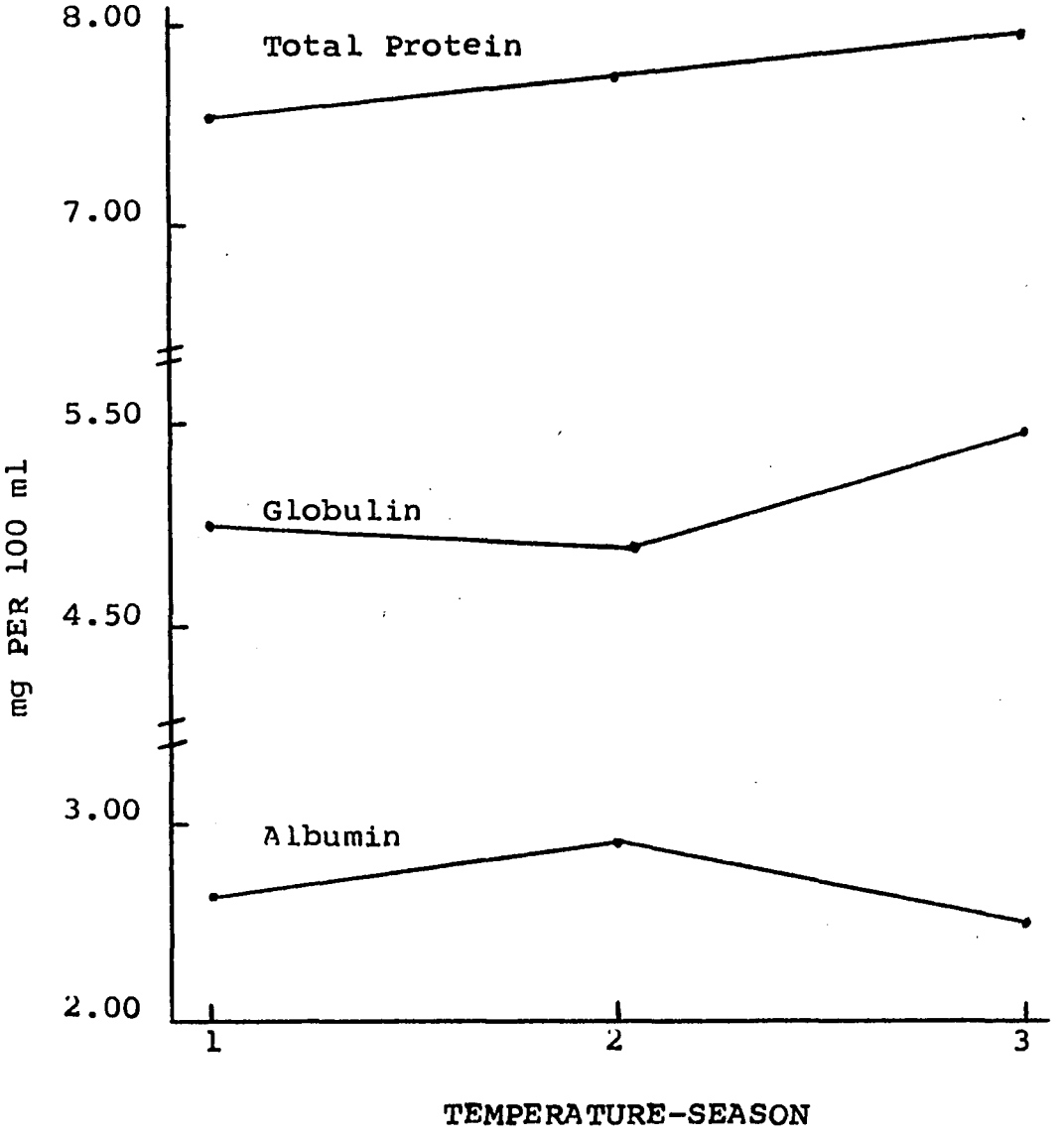


Fig. 11. Relationship between temperature-season and serum total protein, albumin, and globulin.

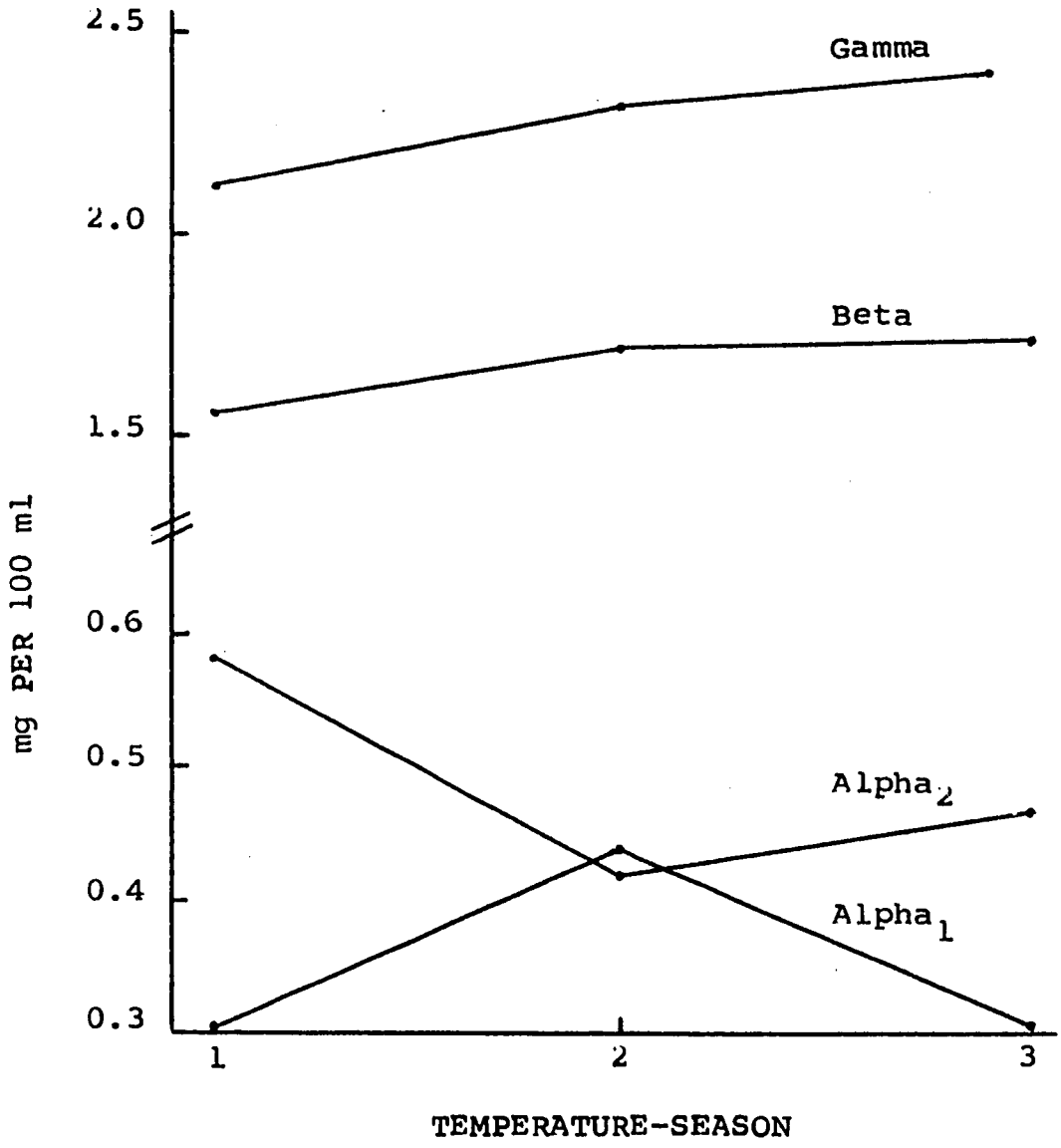


Fig. 12. Relationship between temperature-season and $alpha_1$, $alpha_2$, beta, and gamma globulins.

1.74 mg% in the hot temperature-season. Values for gamma globulin were 2.11, 2.32, and 2.40 mg% for temperature-seasons 1, 2, and 3, respectively. The increases in the gamma fraction were significant ($P<0.05$) and linear.

Figures 11 and 12 show that the slight increase in total protein during temperature-season progression can be accounted for by an increase in the globulins. Furthermore, globulin increases are primarily attributed to an increase in the gamma fraction.

No significant differences were found in blood serum proteins in regard to reproductive status. However, it is to be noted that animals which displayed abnormal reproductive patterns (reproductive status 4 and 5) possessed higher levels of total serum protein (Table 11) than animals with normal reproductive patterns (reproductive status 1, 2, and 3). The mean total serum protein was 7.89 mg% for the two groups with abnormal reproduction patterns versus a mean of 7.48 mg% for the animals in a normal reproductive status. The data presented in Table 12 show that the beta and gamma globulins account for the higher serum protein values for these two reproductive categories.

F. Milk Production

The milk yield per day measured in kilograms of FCM was 16.4, 21.1, and 16.4 for the cool, intermediate and hot temperature-seasons, respectively. The actual

Table 11. Mean serum total protein and relative percents of albumin, and globulin values for five categories of reproductive status.

Reproductive status	Total protein	Albumin	Globulin
	(mg%)	- - - - (%)	- - - -
1	7.30	40.55	59.42
2	7.55	37.65	62.35
3	7.64	36.63	61.37
4	7.92	32.78	67.27
5	7.87	31.70	68.30

Table 12. Relative percents of alpha $alpha_2$, beta, and gamma globulins for five categories of reproductive status.

Reproductive status	$Alpha_1$	$Alpha_2$	Beta	Gamma
	- - - - - - - - - -	(%)	- - - - - - - - -	
1	4.72	6.08	21.94	26.53
2	3.81	6.38	22.00	30.27
3	3.90	7.17	22.87	29.73
4	4.90	5.98	23.89	32.63
5	4.38	6.49	23.54	34.01

1 = Pregnant 1-90 days.
2 = Pregnant 91-180 days.
3 = Open, normal breeder.
4 = Open, anestrus.
5 = Open, 4+ services per conception.

mean milk yield per day was 17.9, 18.7, and 18.7 kilograms for the three respective temperature-seasons. These data are presented in Table 13. Statistical analysis did not reveal any significant season effect on actual milk yield per day nor FCM production per day. A significant ($P<0.05$) effect of reproductive status on milk yield per day was displayed, but this can be accounted for by the fact that there was a great variation in the number of days in lactation among the reproductive categories. The open, normal breeders had a low mean of 67 days in lactation while animals pregnant 91-180 days had the high mean of 240 days in lactation.

A significant ($P<0.05$) linear season effect on actual milk yield per month and a significant ($P<0.01$) linear season effect on FCM production per month were found. These production data corrected for number of days per month generate the plots shown in Figure 13. The monthly actual milk yield decreased from a mean of 580.2 kilograms in the cool temperature-season to a low of 428.2 kilograms in the hot temperature-season (Table 13). On the basis of FCM there was an increase from 511.1 kilograms per month during the cool temperature-season to 524.9 kilograms for the intermediate temperature-season. The hot temperature-season resulted in a depression of the mean monthly FCM production to 480.1 kilograms.

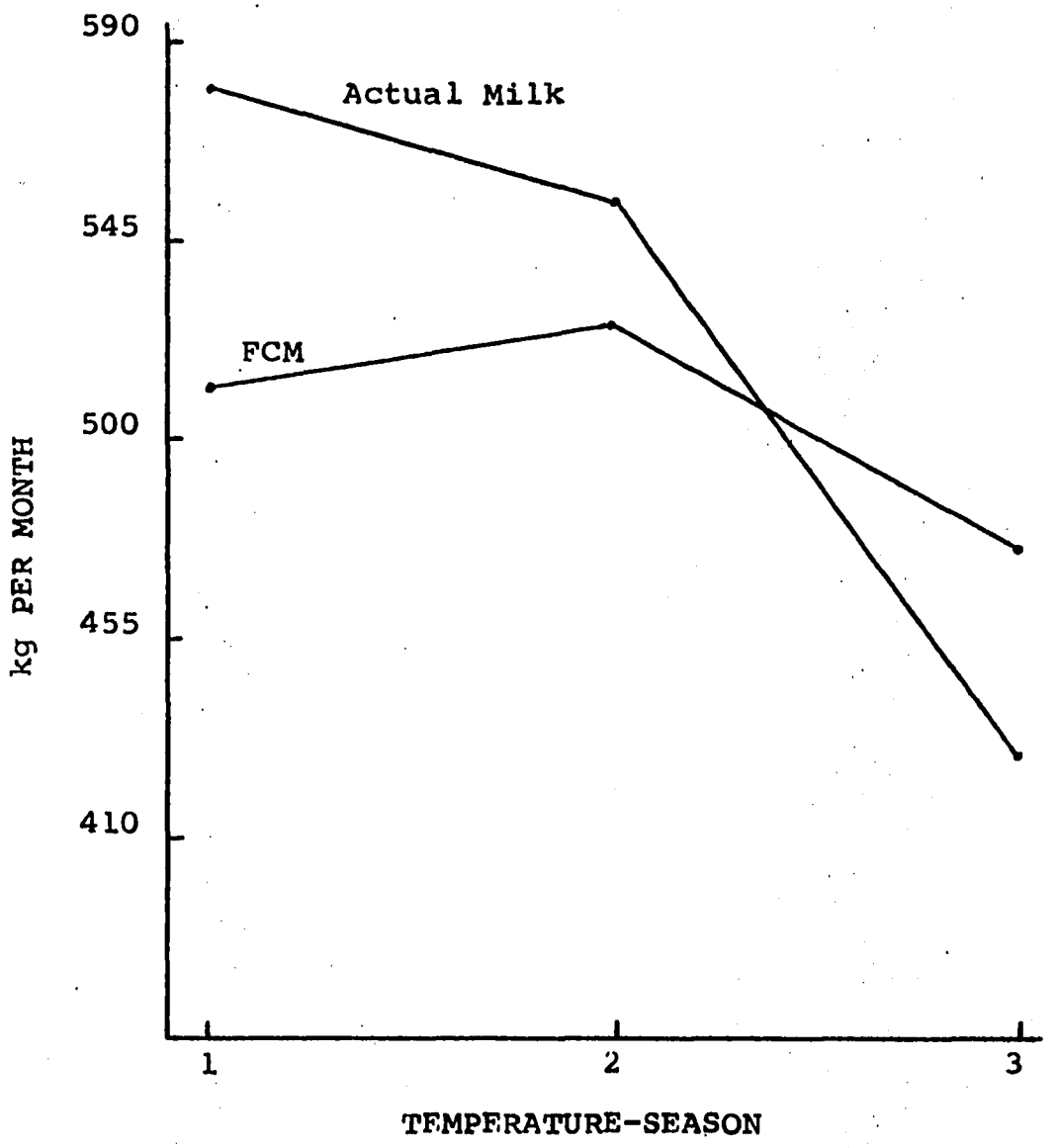


Fig. 13. Relationship between temperature-season and actual milk yield per month and FCM production per month.

Data for milk yield per day were collected on the day of blood collection, whereas the data for monthly milk yield were determined from daily records of milk production. This accounts for the statistical significance being found for monthly milk yield but not for milk yield per day. It also indicates that milk production on the day of blood collection was not a good criterion for true milk production.

Table 13. Mean milk yield for the three temperature-seasons.

Temperature-season	Milk per day	Milk per month	FCM per day	FCM per month
		(kg)		
1-Cool	17.9	580.2	16.4	511.1
2-Intermediate	18.7	553.9	21.1	524.9
3-Hot	18.7	428.2	16.4	480.1

G. Rectal Temperature and Respiration Rate

A significant ($P<0.01$) correlation coefficient of 0.56 was found to exist between rectal temperature and respiration rate in this study.

The mean rectal temperatures and respiration rates for the three temperature-seasons are presented in Table 14. Analysis of variance revealed a significant ($P<0.01$)

linear and quadratic effect on rectal temperature. Mean rectal temperatures of 38.8 and 38.5 C were determined for the cool and intermediate temperature-seasons, respectively. The hot temperature-season resulted in an elevation to 39.6 C. Figure 14 illustrates these data in graphic form.

The data in Table 14 show mean respiration rates of 22.3, 29.0, and 63.7 counts per minute for the cool, intermediate, and the hot temperature-season, respectively. The effect of season on respiration rate was significant ($P<0.01$). The effect was both linear and quadratic as shown in Figure 15.

Table 14. Mean rectal temperature and respiration rate for the three temperature-seasons.

Temperature-season	Rectal temperature	Respiration rate
	(C)	(counts/min)
1-Cool	38.8	22.3
2-Intermediate	38.5	29.0
3-Hot	39.6	63.7

The mean values for rectal temperatures and respiration rate for the five categories of reproductive status are presented in Table 15. Statistical analysis did not show any significant effect of reproductive status on

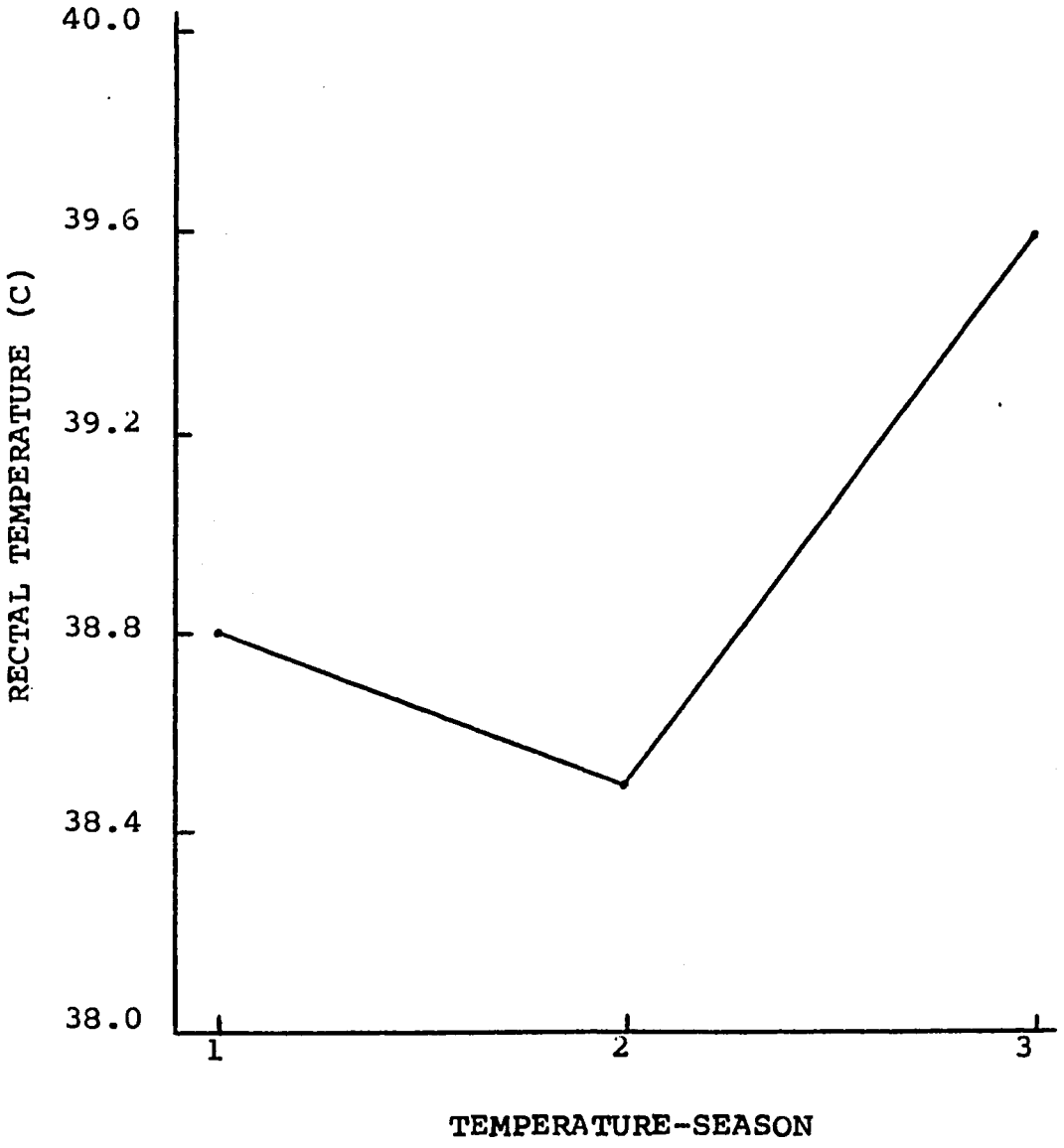


Fig. 14. Relationship between temperature-season and rectal temperature.

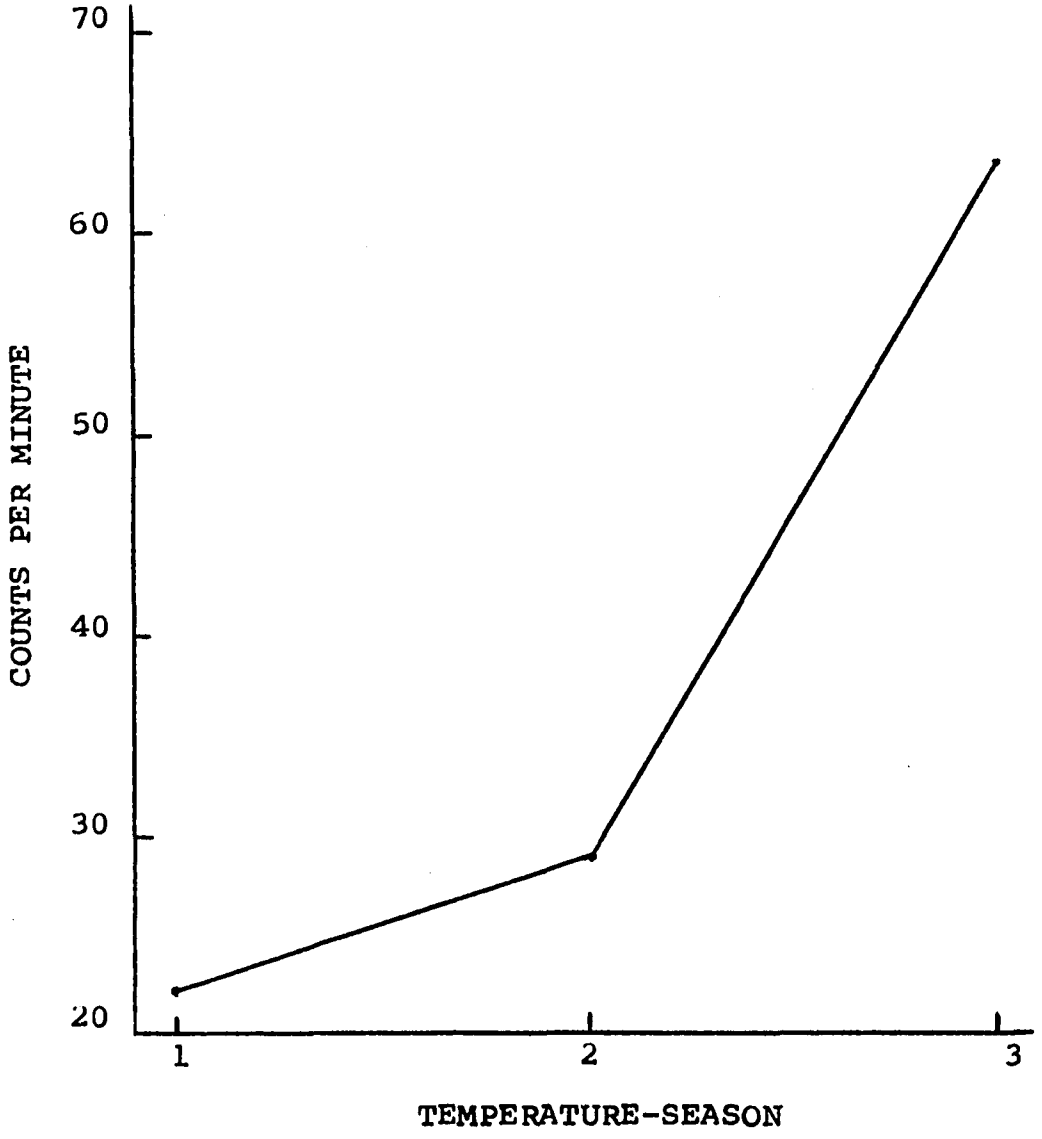


Fig. 15. Relationship between temperature-season and respiration rate.

either of these variables.

Table 15. Mean rectal temperature and respiration rate for the five categories of reproductive status.

Reproductive status	Rectal temperature	Respiration rate
	(C)	(counts/min)
1	38.7	31.7
2	38.9	42.4
3	39.9	35.0
4	39.0	39.0
5	39.2	39.9

1 = Pregnant 1-90 days.
2 = Pregnant 91-180 days.
3 = Open, normal breeder.
4 = Open, anestrus.
5 = Open, regular cycle, 4+ services/conception.

H. Integrating Discussion

In order to fully evaluate the findings of this study, certain interrelating factors must be considered regarding the data obtained. Table 16 presents correlation coefficients relating cortisol level with certain other variables.

A significant ($P<0.05$) correlation coefficient of 0.15 between plasma cortisol level and hematocrit percent was obtained. Furthermore, a significant ($P<0.01$) correlation coefficient of 0.25 was found to exist between

cortisol concentrations and hemoglobin values. However, the experimental design does not allow any cause and effect relationship to be drawn between cortisol level and hematocrit or hemoglobin levels. It may be that the significance of these correlation coefficients should be attributed to a like response to temperature-season in these variables rather than to any co-dependence between them.

Wegner and Stott (83) determined a leukocytosis after ACTH injection in dairy heifers. In this study a leukocytosis was observed as the temperature-season progressed from intermediate to hot conditions but the low correlation coefficient of 0.04 (Table 16) between cortisol level and circulating leukocytes does not imply any significant relationship between these two variables.

It has been reported that lymphocytes are under the control of the adrenal cortex (55). Plasma corticosteroid increases have been associated with a lymphopenia (83). However, Table 16 reveals a positive and significant ($P<0.05$) correlation coefficient of 0.16 between cortisol level and circulating lymphocyte numbers. Similarly, a positive and significant ($P<0.01$) correlation coefficient of 0.19 is shown between cortisol level and lymphocytes expressed as a percent of the total leukocytes. Therefore, the results of this study indicate a lymphocytosis rather

than a lymphopenia associated with increased activity of the adrenal cortex.

Several workers (55, 60, 69, 83) have reported an increase in circulating neutrophils to be associated with increased adrenal cortex activity. However, the data in Table 16 reveal no significant correlation between cortisol and the number of circulating neutrophils. A significant ($P<0.05$) negative correlation of -0.13 between cortisol and percent neutrophils is revealed. Thus, these results indicate that any association between adrenal cortex activity and neutrophil status exists on a basis relative to total leukocytes and is of a negative rather than a positive nature.

A non-significant negative correlation coefficient between cortisol and the number of circulating eosinophils is shown in Table 16. However, eosinophils as a percent of the total leukocytes show a significant ($P<0.05$) negative correlation of -0.16 with cortisol level. Therefore, it is suggested that increased adrenal cortex activity tends to promote an eosinopenia. This confirms reports by other workers (53, 55, 60, 69, 76, 83) who have reported an eosinopenia produced by an increase in circulating glucocorticoids induced by an increase in activity of the pituitary-adrenal axis.

Table 16. Correlation coefficients between plasma cortisol levels and certain blood factors.

Variable	Cortisol
	(r)
Hematocrit	0.15*
Red blood cells	0.08
Hemoglobin	0.25**
Oxyhemoglobin	0.13*
Leukocytes	0.04
Lymphocytes (No.)	0.16*
Neutrophils (No.)	-0.05
Eosinophils (No.)	-0.11
Lymphocytes (%)	0.19**
Neutrophils (%)	-0.13*
Eosinophils (%)	-0.16*

* ($P<0.05$).
** ($P<0.01$).

Table 17 reveals significant ($P<0.01$) negative correlation coefficients of -0.37, -0.22, -0.22, and -0.38 between cortisol and total serum protein, total globulins, $alpha_1$, and gamma globulins, respectively. Significant ($P<0.01$) positive correlation coefficients of 0.25 and 0.18 between cortisol level and albumin and beta globulin levels are shown.

Circulating corticosteroids can be bound to albumin (91). Therefore, it may be postulated that a cause and effect relationship exists between corticosteroid level and blood serum albumin levels. This would account for the significant ($P<0.01$) correlation coefficient of 0.25 between cortisol and albumin and verify this correlation as a cause and effect relationship.

Corticosteroids may also be bound to a globulin (91). Therefore, on the same basis of reasoning, it could be presumed that the beta globulin fraction is related to the cortisol level since a significant ($P<0.01$) and positive correlation coefficient of 0.18 exists between these two variables. The negative correlation coefficients ($P<0.01$) of -0.22, -0.22, and -0.38 between cortisol and total globulins, $alpha_1$, and gamma globulins, respectively (Table 17), should seemingly be attributed to some factor(s) which influences both cortisol and these serum protein fractions.

Table 17. Correlation coefficients between serum proteins, milk yield, rectal temperature, and respiration rate values and plasma cortisol level.

Variable	Cortisol
	(r)
Total serum protein	-0.37**
Albumin	0.25**
Total globulins	-0.22**
$Alpha_1$	-0.22**
$Alpha_2$	0.05
Beta	0.18**
Gamma	-0.38**
Milk yield (daily)	-0.04
Milk yield (monthly)	-0.03
FCM (daily)	0.01
FCM (monthly)	0.02
Rectal temperature	-0.32**
Respiration rate	-0.31**

** ($P<0.01$).

Although not statistically significant, it is noted that animals in the first trimester of pregnancy displayed the highest relative percent of serum albumin (Table 11). Furthermore, these animals possessed the lowest level of total serum protein and the lowest relative percents of total, beta, and gamma globulins (Tables 11 and 12). This observation, coupled with the significant ($P<0.01$) relationships existing between cortisol and these serum protein components, indicates a possible physiological pattern involving adrenal glucocorticoids and these specified serum protein components.

The question of whether or not pregnancy is a normal endocrine state for the female is of both theoretical and practical importance in reproductive physiology. The existence of such a pattern may provide a means of more accurately evaluating the degree of homeostasis in various states of pregnancy. Additionally, such a pattern may become an important adjunct in the evaluation of the effects of hormone therapies which are used for the control of reproductive activities in the human as well as domestic animals.

High levels of milk yield would demand high levels of metabolic activity. Classically, glucocorticoids promote metabolism and would thus be expected to be exhibited in greater concentration at higher levels of milk yield.

However, the correlation coefficients between cortisol concentration and measures of milk production in this study were very low. Daily and monthly actual milk yield showed correlation coefficients with cortisol levels of -0.04 and -0.03, respectively (Table 17). Daily and monthly FCM production correlation coefficients with cortisol were 0.01 and 0.02, respectively.

Therefore, it appears that a need for increased glucocorticoid levels to sustain a high level of metabolism for milk production is not mandatory. Similar findings have been reported by Bergman (4) for lactating cows. MacAdam and Eberhart (39) noted an increase in circulating corticosteroid level in conjunction with lactation, but their work involved a relatively short term experiment of four days.

It may be suggested that a high level of milk production can be a metabolic stress and the calorogenic effect of the glucocorticoids tends to magnify this stress. This would preclude an elevation of glucocorticoids so that physiological homeostasis could be maintained.

Values for rectal temperature and respiration rate showed significant ($P<0.01$) negative correlation coefficients of -0.32 and -0.31, respectively, with circulating cortisol concentration (Table 17). Alvarez and Johnson (2) also reported a negative correlation coefficient of -0.35 between

rectal temperature and glucocorticoid concentration. The negative relationship between cortisol level and rectal temperature and respiration rate, which are indicative of heat stress, supports the supposition that the calorogenic effect of the glucocorticoids is an important factor in regard to adrenal cortex function in the lactating bovine.

It is classically accepted that secretions from the adrenal cortex are stimulated by a release of pituitary ACTH in response to any stress conditions tending to disrupt physiological homeostasis. However, the results of this study suggest the presence of some other physiological mechanism which is capable of altering ACTH release or ACTH regulation of the adrenal cortex. The exact nature and mode of action of such a control mechanism remains unknown pending additional research.

V. SUMMARY AND CONCLUSIONS

This investigation was undertaken in an effort to determine the effects of ambient conditions on adrenal cortical and other physiological functions in the lactating bovine over a relatively long period of time. Also, this work was designed to study the interrelationships of adrenal cortex function with certain other physiological variables.

Data were collected from lactating Holstein cows over a period of one calendar year. The year was divided into cool, intermediate, and hot temperature-seasons. Eighteen to 24 animals, with an average of 22, were utilized each month for sample collection. This resulted in the data being comprised of a total of 264 cow-months.

Circulating cortisol level was used to evaluate adrenal cortex function. Other measurements included hematocrit values, circulating red blood cell numbers, leukocyte profile, hemoglobin and oxyhemoglobin levels, concentration of blood serum protein, protein fractions, milk production, rectal temperatures, and respiration rates.

A mean plasma cortisol concentration of 42.4 ng/ml was found for the cool temperature-season. The cortisol

concentration declined in a linear fashion to a mean of 22.8 ng/ml in the hot temperature-season. These data suggest that the adrenal cortex function was not controlled by an increased ACTH response to combat heat stress in the warmer months, but that the animals were able to adjust physiologically to heat stress and thereby minimize glucocorticoid requirements. This would seemingly correspond with the stage of resistance of the general adaptation syndrome. It is further suggested that increased ambient and metabolic heat loads may trigger a depression of adrenal glucocorticoid secretion thereby offsetting the calorogenic effect of these hormones.

In regard to reproductive status, animals in the first 90 days of pregnancy showed the highest values of circulating cortisol levels with a mean of 47.5 ng/ml. The lowest values were found in animals pregnant 91-180 days which displayed a mean of 29.1 ng/ml. Non-pregnant animals had mean circulating cortisol levels ranging from 30.8 to 33.3 ng/ml. However, differences in circulating cortisol levels for the various categories of reproductive status did not show statistical significance.

A significant ($P<0.01$) negative correlation coefficient (-0.32) between circulating cortisol concentration and animal age was displayed. Also, a significant ($P<0.01$) negative correlation coefficient (-0.31) was determined

between cortisol level and animal lactation number. It is suggested that the effect of lactation number was not a primary effect, but existed because lactation number is a reflection of animal age.

The data reveal that measurements of climatic conditions indicative of ambient heat stress were associated with a depression of hematocrit, red blood cells, hemoglobin, and oxyhemoglobin values. It is suggested that a depression of these factors is related to a reduction in cellular oxygen requirements in an effort to reduce metabolic heat load to compensate for an elevated environmental heat load.

A significant ($P<0.05$) effect of reproductive status on red blood cell numbers existed. Animals in the first 90 days of pregnancy showed the highest number of circulating red blood cells (775.6 cells/cmm). The lowest numbers were found for open, anestrus animals (689.7 cells/cmm). Animals pregnant 91-180 days, non-pregnant normal breeders, and cycling problem breeders displayed intermediate numbers of circulating red blood cells.

No statistically significant differences were found for hematocrit, hemoglobin, and oxyhemoglobin values for the various categories of reproductive status. However, the values for these constituents followed the same general trend as the numbers of circulating red blood cells.

The greatest number of total circulating leukocytes was found in the cool temperature-season. Progression from the intermediate to hot temperature-season elicited evidence of a leukocytosis. Changes observed in total leukocytes can be primarily attributed to changes in the number of circulating neutrophils. Progression from the intermediate to hot temperature-season produced an eosinophilia.

Open, normal breeders displayed a greater number of total circulating leukocytes, lymphocytes, and neutrophils than did animals in the other categories of reproductive status. However, the lowest number of circulating eosinophils was found for open, normal breeders while animals pregnant 91-180 days showed the highest number.

Warmer seasonal conditions promoted an increase in total serum protein values. This increase can be attributed to the globulins and primarily to the gamma globulin fraction.

It was noted that animals displaying abnormal reproductive patterns possessed higher levels of total serum protein than animals with normal reproductive patterns. These differences were primarily attributed to the beta and gamma globulins. However, the differences were not statistically significant.

Temperature-season had a significant ($P<0.05$) linear effect on actual milk yield and a significant ($P<0.01$) linear

effect on FCM production on a monthly basis corrected for the number of days per month. The highest level of actual milk and FCM production was found in the intermediate temperature-season. The lowest level was found in the hot temperature-season. A significant ($P<0.05$) effect of reproductive status on milk yield was obtained. However, this is explained by the fact that days in lactation were a reflection of the reproductive status.

Both rectal temperature and respiration rate were elevated during the hot-temperature-season ($P<0.01$), indicating that a heat stress was associated with that season. A significant ($P<0.01$) correlation coefficient of 0.56 was found to exist between rectal temperature and respiration rate. Reproductive status exhibited no discernible pattern on rectal temperature or respiration rate.

A significant ($P<0.05$) correlation of 0.15 was found to exist between plasma cortisol level and hematocrit value. A significant ($P<0.01$) correlation coefficient of 0.25 was found between cortisol concentration and hemoglobin value. However, a like response of these variables to climatic conditions may have been responsible for these significant correlations rather than any intrinsic relationship between them.

Increased adrenal cortex activity, as indicated by plasma cortisol concentration, was correlated with a

lymphocytosis. However, there was a decrease in the percent of circulating neutrophils as well as a relative eosinopenia associated with increased cortisol concentration.

The data revealed an apparent negative association between cortisol level and total serum protein, total globulin, alpha$_1$, and gamma globulins. However, cortisol level was positively associated with serum albumin, alpha$_2$, and beta globulin. It may be postulated that a cause and effect relationship exists between cortisol and albumin and beta globulin.

Under the conditions of this study, there was no discernible demand for glucocorticoids to sustain milk production. It is suggested that high glucocorticoid levels, because of their calorogenic effect, might hinder rather than enhance the physiological homeostasis of lactating animals.

The circulating cortisol concentration was depressed by conditions of ambient heat stress which elicited increasesin both rectal temperature and respiration rate. This tends to support the postulation that the calorogenic effect of glucocorticoids may be detrimental rather than beneficial to adaptation and maintenance of homeostasis under conditions of heat stress over a relatively long period of time.

The findings of this investigation appear to justify the following summary statements:

1. Adrenal cortex function, as indicated by circulating cortisol levels, is depressed by hot ambient conditions of relatively long duration, which tend to produce heat stress.
2. Prolonged heat stress produces a depression of hematocrit values, red blood cell numbers, hemoglobin and oxyhemoglobin values.
3. The numbers of circulating red blood cells is elevated in cows in the first 90 days of pregnancy and depressed in non-pregnant, anestrus animals.
4. Progression from an intermediate to a hot temperature-season promotes a leukocytosis, primarily attributed to changes in the number of circulating neutrophils. This seasonal progression also promotes an eosinophilia.
5. Warmer seasonal conditions promote an increase in total serum protein concentration with the gamma globulin fraction being primarily affected.
6. Seasonal heat stress can impede the efficiency of milk production.

7. Lymphocytosis, a decrease in the percent of circulating neutrophils, and a relative eosinopenia are indicative of increased adrenal cortex activity.
8. A co-dependence may exist between circulating cortisol level and albumin and beta globulin blood protein fractions.
9. Milk production does not demand a subsequent elevation in glucocorticoid levels.
10. Additional research is needed to further elucidate mechanism(s) of adrenal cortex control under prolonged conditions of heat stress.

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VII. APPENDICES

Appendix table 1. Summary of the number of experimental animals by age, lactation number, reproductive status, and temperature-season.

Age (years)	No. of animals*	Lact. No.	No. of animals	Reprod. status	No. of animals	Temp.-season	No. of animals
2	55	1	84	1	36	1	90
3	46	2	67	2	26	2	87
4	57	3	50	3	30	3	87
5	48	4	26	4	88		
6	17	5	11	5	84		
7	6	6	14				
8	12	7	12				
9	18						
10	5						

*Animal samples

Appendix table 2. Mean hematocrit, red blood cells, hemoglobin, and oxyhemoglobin values for the three temperature-seasons.

Temperature-season	Hematocrit	Red blood cells	Hemoglobin	Oxyhemoglobin
	%	(cells/cmm)	(g/100ml)	(g/100ml)
1-Cool	35.9	726	10.3	9.4
2-Intermediate	36.0	743	10.5	10.2
3-Hot	34.0	708	9.9	9.4

Appendix table 3. Mean relative percents of lymphocytes, neutrophils, and eosinophils for the three temperature-seasons.

Temperature-season	Lymphocytes	Neutrophils	Eosinophils
		(%)	
1-Cool	55.8	37.6	5.4
2-Intermediate	54.6	35.5	6.2
3-Hot	52.2	39.1	7.5

Appendix table 4. Mean serum total protein, albumin, and globulin values for the three temperature-seasons.

Temperature-season	Total Protein	Albumin	Globulin
	(mg%)		
1-Cool	7.54	2.63	4.99
2-Intermediate	7.75	2.86	4.89
3-Hot	7.97	2.50	5.47

Appendix table 5. Mean serum $alpha_1$, $alpha_2$, beta, and gamma globulin values for the three temperature-seasons.

Temperature-season	$Alpha_1$	$Alpha_2$	Beta	Gamma
		(mg%)		
1-Cool	0.30	0.58	1.55	2.11
2-Intermediate	0.44	0.42	1.72	2.32
3-Hot	0.31	0.47	1.74	2.40

Appendix table 6. Mean red blood cells, total leucocytes, lymphocytes, neutrophils, and eosinophils for the five categories of reproductive status.

Reproductive status	Red blood cells	Total leucocytes	Lympho-cytes	Neutro-phils	Eosino-phils
			(cells/cmm)		
1	776	9,633	4,301	3,085	628
2	725	9,085	4,313	3,165	768
3	724	10,742	5,802	4,303	476
4	690	9,975	5,372	3,945	573
5	742	9,984	5,969	3,651	580

1 = Pregnant 1-90 days.
2 = Pregnant 91-180 days.
3 = Open, normal breeder.
4 = Open, anestrus.
5 = Open, 4+ services per conception.

Appendix table 7. Mean relative percents of lymphocytes, neutrophils, and eosinophils for the five categories of reproductive status.

Reproductive status	Lymphocytes	Neutrophils	Eosinophils
	- - - - - - - - - - - - - - (%) - - - - - - - - - - - - - -		
1	51.3	37.8	7.4
2	53.2	35.1	8.9
3	53.8	39.1	5.1
4	54.3	37.4	6.0
5	55.8	37.3	5.9

1 = Pregnant 1-90 days.
2 = Pregnant 91-180 days.
3 = Open, normal breeder.
4 = Open, anestrus.
5 = Open, 4+ services per conception.

Appendix table 8. Mean serum albumin, globulins, alpha$_1$, alpha$_2$, beta, and gamma globulin values for the five categories of reproductive status.

Reproductive status	Albumin	Globulin	Alpha$_1$	Alpha$_2$	Beta	Gamma
			(mg%)			
1	2.96	4.34	0.35	0.44	1.60	1.94
2	2.84	4.71	0.29	0.48	1.66	2.29
3	2.79	4.69	0.30	0.55	1.75	2.27
4	2.60	5.33	0.39	0.47	1.89	2.58
5	2.49	5.38	0.35	0.51	1.85	2.68

1 = Pregnant 1-90 days.
2 = Pregnant 91-180 days.
3 = Open, normal breeder.
4 = Open, anestrus.
5 = Open, 4+ services per conception.

Appendix table 9. Mean milk per day, milk per month, FCM per day, FCM per month, and days in lactation data for the five categories of reproductive status.

Reproductive status	Milk per day	Milk per month	FCM per day	FCM per month	Days in lactation
	(kg)				(No.)
1	14.5	461.0	13.3	423.4	196
2	12.0	361.6	11.6	350.5	240
3	20.0	581.5	18.1	522.4	67
4	20.6	607.3	21.8	549.2	108
5	19.2	575.7	17.8	532.3	149

1 = Pregnant 1-90 days.
2 = Pregnant 91-180 days.
3 = Open, normal breeder.
4 = Open, anestrus.
5 = Open, 4+ services per conception.

Appendix table 10. Analyses of variance F-values for cortisol, hematocrit, and red blood cells.

Variance source	d.f.	Cortisol	Hemato-crit	RBC
		- - - - (F-value) - - - -		
Within	203			
Total reduction	34	39.438	1.445	1.541
Season	2	138.002**	4.072*	1.926
Linear	1	275.785**	7.134**	0.213
Quadratic	1	0.219	1.011	3.640
Reproduction	4	0.534	1.787	2.939*
Linear	1	0.338	0.209	0.196
Quadratic	1	0.618	5.784	11.436**
Cubic	1	0.007	0.525	0.098
Quardic	1	1.175	0.628	0.027
Lactation	6	0.448	1.008	0.982
Season x Reproduction	8	0.952	0.579	1.639
Season x Lactation	12	0.598	1.277	0.496
Lactation x Linear	1	11.719**	1.075	2.812
Age x Linear	1	0.709	0.840	2.717
Error	169			

* ($P<0.05$).
** ($P<0.01$).
RBC = Red blood cells.

Appendix table 11. Analyses of variance F-values for hemoglobin, oxyhemoglobin, and total leucocytes.

Variance source	d.f.	Hemoglobin	Oxyhemoglobin	Total leucocytes
		- - - - - -	(F-value)	- - - - -
Within	203			
Total reduction	34	1.286	1.366	0.595
Season	2	1.090	1.031	0.050
Linear	1	0.276	0.103	0.000
Quadratic	1	1.904	1.959	0.099
Reproduction	4	0.771	2.015	0.444
Linear	1	1.102	0.628	0.067
Quadratic	1	1.528	2.451	1.687
Cubic	1	0.001	4.295	0.022
Quardic	1	0.015	0.687	0.000
Lactation	6	0.817	0.762	0.061
Season x Reproduction	8	1.049	1.125	0.882
Season x Lactation	12	0.725	1.162	0.745
Lactation x Linear	1	1.412	0.056	0.144
Age x Linear	1	2.111	2.088	0.018
Error	169			

Appendix table 12. Analyses of variance F-value for lymphocytes (No.), neutrophils (No.), and eosinophils (No.)

Variance source	d.f.	Lymphocytes (No.)	Neutrophils (No.)	Eosinophils (No.)
		- - - - - (F-value) - - - - -		
Within	203			
Total reduction	34	0.873	1.142	1.550
Season	2	2.479	0.186	1.349
Linear	1	4.031	0.000	2.589
Quadratic	1	0.928	0.371	0.109
Reproduction	4	2.256	2.156	0.932
Linear	1	4.538	0.876	0.373
Quadratic	1	3.184	6.574	0.118
Cubic	1	0.603	0.808	0.082
Quardic	1	0.698	0.366	3.154
Lactation	6	0.489	0.488	0.673
Season x Reproduction	8	0.634	0.595	0.781
Season x Lactation	12	0.762	1.116	1.327
Lactation x Linear	1	0.042	1.035	3.045
Age x Linear	1	0.107	0.106	0.588
Error	169			

Appendix table 13. Analyses of variance F-values for lymphocytes (%), neutrophils (%), and eosinophils (%).

Variance Source	d.f.	Lympho-cytes (%)	Neutro-phils (%)	Eosino-phils (%)
		- - - - - - (F-value) - - -		
Within	203			
Total reduction	34	1.294	1.077	1.610
Season	2	2.404	1.740	1.299
Linear	1	4.801	2.788	2.485
Quadratic	1	0.008	0.691	0.112
Reproduction	4	1.819	1.819	0.849
Linear	1	2.470	1.857	0.716
Quadratic	1	2.700	2.205	0.062
Cubic	1	1.589	0.829	0.001
Quardic	1	0.518	2.386	2.617
Lactation	6	0.394	0.443	0.450
Season x Reproduction	8	0.877	0.503	0.708
Season x Lactation	12	1.073	0.816	1.505
Lactation x Linear	1	1.049	0.400	1.588
Age x Linear	1	0.063	0.411	0.412
Error	169			

Appendix table 14. Analyses of variance F-values for total serum protein, serum albumin, and serum globulins.

Variance source	d.f.	Total serum protein	Serum albumin	Serum globulin
		- - - - (F-value) - - - -		
Within	203			
Total reduction	34	1.408	1.246	1.230
Season	2	0.288	1.479	1.524
Linear	1	0.041	1.951	2.010
Quadratic	1	0.536	1.006	1.037
Reproduction	4	1.225	0.315	0.360
Linear	1	0.080	0.708	0.765
Quadratic	1	0.002	0.090	0.141
Cubic	1	3.809	0.258	0.339
Quardic	1	1.010	0.205	0.195
Lactation	6	0.149	1.217	1.226
Season x Reproduction	8	0.878	0.561	0.560
Season x Lactation	12	1.084	1.479	1.415
Lactation x Linear	1	0.448	0.957	1.081
Age x Linear	1	4.608*	1.256	1.102
Error	169			

* ($P<0.05$).

Appendix table 15. Analyses of variance F-values for $alpha_1$, globulin, $alpha_2$, globulin, and beta globulin.

Variance source	d.f.	$Alpha_1$ globulin	$Alpha_2$ globulin	Beta globulin
		- - - - (F-value) - - - - -		
Within	203			
Total reduction	34	0.990	1.497	1.799
Season	2	0.241	0.851	0.601
Linear	1	0.419	1.213	0.764
Quadratic	1	0.063	0.488	0.437
Reproduction	4	0.404	0.195	1.076
Linear	1	0.198	0.280	0.012
Quadratic	1	0.470	0.085	0.645
Cubic	1	0.133	0.330	3.196
Quardic	1	0.814	0.086	0.450
Lactation	6	0.153	0.258	0.411
Season x Reproduction	8	0.177	0.650	1.845
Season x Lactation	12	0.449	0.623	1.591
Lactation x Linear	1	3.035	6.591	1.784
Age x Linear	1	1.013	0.264	2.357
Error	169			

Appendix table 16. Analyses of variance F-values for gamma globulin, milk production per day, and milk production per month.

Variance source	d.f.	Gamma globulin	Milk production per day	Milk production per month
		- - - - - - - (F-value) - - - -		
Within	203			
Total reduction	34	2.099	8.423	6.907
Season	2	3.553*	0.072	3.030*
Linear	1	7.105**	0.143	6.050**
Quadratic	1	0.002	1.304	0.009
Reproduction	4	0.410	2.324*	2.249
Linear	1	0.014	0.441	0.569
Quadratic	1	0.001	0.440	0.761
Cubic	1	1.485	6.217**	6.245
Quardic	1	0.141	2.196	1.421
Lactation	6	1.873	0.052	0.149
Season x Reproduction	8	0.626	0.866	1.811
Season x Lactation	12	0.689	1.664	1.621
Lactation x Linear	1	0.039	53.470**	41.060**
Age x Linear	1	0.002	0.393	0.070
Error	169			

* ($P<0.05$).
** ($P<0.01$).

Appendix table 17. Analyses of variance F-values for FCM production per day, and FCM production per month.

Variance source	d.f.	FCM production per day	FCM production per month
		- - - (F-value) - - - -	
Within	203		
Total reduction	34	0.709	6.098
Season	2	0.148	4.648**
Linear	1	0.051	9.203**
Quadratic	1	0.244	0.093
Reproduction	4	0.432	1.288
Linear	1	0.245	1.294
Quadratic	1	1.133	0.015
Cubic	1	0.292	2.058
Quardic	1	0.058	1.784
Lactation	6	0.005	0.058
Season x Reproduction	8	0.111	1.209
Season x Lactation	12	0.578	1.531
Lactation x Linear	1	2.141	43.959**
Age x Linear	1	0.513	1.066
Error	169		

** ($P<0.01$).

Appendix table 18. Analyses of variance F-values for rectal temperature, and respiration rate.

Variance source	d.f.	Rectal temperature	Respiration temperature
		- - - (F-value) - - - -	
Within	203		
Total reduction	34	4.691	15.281
Season	2	19.304**	61.680**
Linear	1	27.969**	103.181**
Quadratic	1	10.639**	20.179**
Reproduction	4	0.505	0.545
Linear	1	1.835	0.841
Quadratic	1	0.000	0.821
Cubic	1	0.099	0.511
Quardic	1	0.086	0.008
Lactation	6	0.258	0.172
Season x Reproduction	8	2.404	1.626
Season x Lactation	12	2.082	0.574
Lactation x Linear	1	0.095	0.028
Age x Linear	1	0.343	9.622**
Error	169		

** ($P<0.01$).

VITA

Jerry Aldrus Lee was born January 22, 1941, in New Orleans, Louisiana. He graduated from Amite High School, Amite, Louisiana, in May of 1959.

In September of 1959, he entered Southeastern Louisiana College and completed the requirements there in August of 1963 for the degree of Bachelor of Science in Animal Science. During his undergraduate work he was active in the Southeastern Louisiana College Gleaner's Club and served as Vice-president and President of that organization.

In June of 1962 he was married to the former Miss Patricia Lautier of Baton Rouge, Louisiana.

He entered the Louisiana State University Graduate School in June, 1965 and received the degree of Master of Science in Physiology and Endocrinology in the Department of Dairy Science in May, 1968. The title of his Master of Science thesis was "Effects of Shade Versus Sun on Adrenal Cortical Function and Metabolism of Lactating Dairy Cattle During Hot Weather."

In June, 1968 he began work toward the degree of Doctor of Philosophy in Physiology and Endocrinology in the Department of Dairy Science. During this period of

graduate study he was elected to membership in Gamma Sigma Delta and to associate membership in the Society of Sigma Xi. He served as an Associate in the Department of Dairy Science from February, 1970 through June, 1971, at which time he resigned to concentrate on the completion of the requirements for the degree of Doctor of Philosophy.

EXAMINATION AND THESIS REPORT

Candidate: Jerry Aldrus Lee

Major Field: Dairy Science (Physiology)

Title of Thesis: Adrenal cortical and other physiological responses to environmental changes in the bovine

Approved:

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

Date of Examination:

April 24, 1973